

Where next on strategic arms?

The nuclear powers should start worrying about next year's review conference of the Non-Proliferation Treaty. The other members will expect much of them.

THE nuclear powers will be in a serious jam this time next year. Then, they will no longer be able to put at the backs of their minds the knowledge that the third review conference of the Non-Proliferation Treaty (NPT) will be just a few months ahead. They will instead be haunted by bad memories of how the last conference broke up in August 1980 with the non-nuclear signatories of the treaty hurling recriminations at the Soviet Union, the United Kingdom and the United States (China and France do not even belong) for their failure to make progress towards the control of strategic arms. Heaven forbid another rough-house will be the cry a year from now. But in reality, in 1980 the nuclear powers had quite a lot to say. Since the previous conference in 1975, they had negotiated agreements to restrict the use of nuclear weapons for peaceful purposes and, more important, the complex of agreements known collectively as Salt II which restrict the numbers of strategic weapons the Soviet Union and the United States may deploy. True, neither agreement had been ratified by the governments concerned in 1980 (and neither has been even now), but surely, it must have seemed safe to argue, the fact that the agreements have been signed must be evidence of "good faith", the spirit in which NPT requires the superpowers to negotiate? In the event, that argument cut no ice. The nuclear powers were sent away with as clear a warning as they could have had that NPT could collapse if nothing happened on the control of strategic nuclear weapons. So, this time next year, the nuclear powers will be woefully aware that in 1985 they will have even less to say than in 1980.

What has gone wrong? The Reagan Administration was slow to square its electoral rhetoric with its treaty obligations to negotiate on strategic arms, but eventually two parallel sets of talks were begun in 1982, on European missiles and on strategic missiles, both at Geneva. The first set of talks (called INF for Intermediate Nuclear Forces) broke down last November, when the Soviet delegates walked out of the Geneva negotiations in protest at the deployment of cruise and Pershing II missiles in Western Europe. Against expectations, the strategic talks (called START for Strategic Arms Reduction Talks) ran their planned course, but Mr Konstantin Chernenko, the new Soviet leader, keeps saying (most recently last weekend) that the talks are now suspended until cruise and Pershing missiles are removed from Europe. Since the impasse that has arisen will certainly persist until after the November election in the United States, but since both the nuclear superpowers want NPT to survive, there is a chance that this time next year they will have a common cause in putting together some tale they can tell the other members.

Solutions

What can they say or, better, do? Each will wish to persuade the non-nuclear NPT members that the other is to blame. That temptation should be resisted not only because mass defections from NPT will be no more tolerable if one or the other is thought to be responsible but also because the apportionment of blame conceals an important stumbling block to agreement on both INF and START. Long before the collapse of INF, the Soviet Union had argued that the British and French nuclear forces should be counted in the European balance of forces.

The point has some force. While the British and French Governments have built their nuclear forces for reasons which are

to them strategic — ultimately to provide an element of deterrence against threats to national survival — the missiles could also be aimed at targets within 1,000 km of the East-West German border exactly as if they were SS20s or Pershing IIs. The same difficulty has arisen previously, and it is now said that the British and French forces are tacitly allowed for in the Salt II treaty by means of the less stringent numerical limits with which Soviet missile forces must comply. What seems not to have been appreciated, during the long months at Geneva, is that the SS20 missiles are in some respects exactly comparable with the British and French nuclear forces. Although apparently designed for use against military targets such as troop concentrations, there is nothing to prevent their use against cities in Western Europe (and, because they are mobile, in the Far East). So the SS20s are both squarely within the ambit of INF and, because they supplement other Soviet strategic forces, also part of the START negotiations. The declaration by the North Atlantic Treaty Organization (NATO) in December 1979 that the appearance of SS20s within range of Western Europe would be met by the installation of Pershing II and cruise missiles could well have gone further and have protested that the appearance of the SS20s on the European scene was a breach of the Salt II agreement.

Pitfalls

The only moral in this sorry tale is that nuclear arms control is indivisible. If the two superpowers agree about some narrow aspect of the problem, the consequence will be a tendency for nuclear arms to pop up in greater numbers elsewhere, as they have now done in Europe, East and West. The case for separate talks at Geneva (while they lasted) was that the hope of winning agreement must be greater on a more restricted front. What should now be recognized is that such ambitions are certain to be fruitless. Whichever side is to blame for what has happened, there is everything to be said for mounting the next set of talks in a wider framework that will take account of unambiguously strategic superpower missiles as well as those held by the British and the French and otherwise sited in Europe.

But of what value is this conclusion if the two nuclear superpowers will not talk to each other? Fortunately, the hard work that needs to be done against this time next year does not require that there should be formal negotiations under way at some central location on neutral ground. The standard form on those occasions, anyway, is that people meet for a couple of hours twice a week. No purpose other than symbolic would be served by engaging senior public servants in a continuation of this charade until there is something for them to get their teeth into. A general understanding in Western Europe and North America that the nuclear problem is all of a piece would be a good beginning, and need not wait for the US presidential election in November. There is even, of course, a possibility that a different basis for some future set of talks might simplify the task of persuading the Soviet Union to talk again about the need for an agreement. Since the prospect of an agreement on a comprehensive test-ban, never bright, will be slim at least until the future of major missile systems in the United States has been decided, this is the best hope that the superpowers have of avoiding another drubbing in 1985. Is it really necessary to wait another year before waking up to what will then seem an urgent truth? □



New private monopoly

The British Government is turning its public telephone monopoly into a private ogre.

THE British Government has landed itself in a serious conflict of interest. For more than two years, the government has been fighting through the British Parliament a bill to sell off British Telecom, the public monopoly that now provides telephone services in Britain. The government's motives are mostly laudable. It reckons that a telephone service which is a public company mostly owned by private individuals would be bound to conduct its affairs efficiently, returning a good profit in the ordinary way to its shareholders. That is true. So is the belief that the general public interest will be served by circumscribing the long-standing and much-used right of the public monopoly to be the sole supplier of the terminal equipment for all rented telephone lines. Some moves have already been made in that direction, and more are promised. But the British Government is also anxious that the sale of shares in British Telecom, due to take place within a year, should be a success in the commercial sense. The Chancellor of the Exchequer is counting on the proceeds to help reduce the government's need to borrow money from the financial markets in the years ahead, perhaps to the tune of £4,000 million. The trouble is that the last objective, prudent though it is, sharply conflicts with the objective that the market for the supply of telecommunications services should be genuinely competitive.

The strains are already shockingly apparent. For the past two years, British Telecom has been working a transitional arrangement for administering the approval of terminal equipment other than its own that may be connected to the public network. Technically, suppliers of alternative equipment have been required to submit items of equipment for approval to the Department of Trade and Industry, the government department in charge of telecommunications, which has then taken technical advice from none other than British Telecom, the organization most directly damaged by alternative sources of supply. Is it any wonder that approvals for alternative pieces of terminal equipment have been slow to trickle through? Or that alternative terminal equipment for which approval was withheld should have turned up among the items offered for sale by newly go-go British Telecom? (One such item is British Telecom's "Easa-Phone", essentially a National Panasonic product for whose independent sale a private company failed to get permission more than a year ago.)

Now the regime has changed, in that the British Standards Institution has published a complicated set of standard specifications for terminal equipment, and the business of licensing has been hived off to a nominally independent board, while under the new legislation there will be a new kind of regulatory body (called "OfTel") to supervise the public monopoly when it goes private. The obvious snag is that OfTel's powers to control British Telecom when it has become a private company and a majority of its shares sold to private persons will be puny in comparison with those thought necessary elsewhere, in the United States for example. Thus while British Telecom is exhorted by its draft operating licence not to use the revenues from one service to subsidize another, innocence or guilt in this respect will be harder to establish when British Telecom is a private entity. No wonder that even Lord Weinstock, chairman of the General Electric Company (not to be mistaken for GE of the United States) and until recently the British Government's favourite industrialist, has been saying that the new legislation is simply a way of replacing a public by a private monopoly.

The government's dilemma is that it will not get the whole of its £4,000 million for the sale of shares if it allows British Telecom to be exposed to the full force of commercial competition for terminal equipment — not just handset and answering machines, but branch exchanges, computer equipment and the like. The calculation is probably correct but is also shortsighted. In the last complete financial year (to March 1983) the telephone system had revenues of £6,414, out of which it financed two-thirds of its

capital development and yet managed to turn in a profit of nearly £1,000 million. (In the year just ended, the profits will be less because the nascent company is taking every chance to write off capital assets in advance of going public.) The business about to be sold to the public, in other words, is immensely profitable in commercial terms, charging so much for its services that it can make a handsome profit and at the same time provide the resources that will earn still larger profits in the future. It is in retrospect ironical that this practice has been forced on British Telecom by the British Government's past insistence that the telephone services should not add to its borrowing requirement, but as a consequence the shares soon to be sold will be among the best bargains the stock exchanges have seen for many years, making gentle nonsense of the government's promise that OfTel will not allow charges to increase as quickly as inflation for the next five years. But if, as seems possible, as much as a third of British Telecom's revenue may represent money that could be raised in other ways (by borrowing or raising further equity), it cannot be sensible that the Chancellor of the Exchequer should institutionalize a monopoly on such a scale for the sake of being able to sell a half-share in it for £4,000 million. □

Bad news for Unesco

The British shot across Unesco's bows is more direct than the earlier US threat to withdraw.

Mr Timothy Raison, Minister of State at the British Overseas Development Administration, writes a better letter than Mr George Shultz, US Secretary of State. That is one conclusion to be drawn from a comparison of the letters the two politicians have been sending to Mr Amadou-Mahtar M'Bow, the director general of the United Nations Educational, Scientific and Cultural Organization (Unesco). Mr Shultz's letter last December announcing the still-intended withdrawal of the United States, went out of its way to say that the United States had "no complaint" about the director-general's administration of Unesco — and in the process made the proposed withdrawal less easily intelligible, and cast down the spirits of those working for the organization, many of whom consider that their employer is in a muddle, intellectual and administrative. Mr Raison's letter, earlier this month, should help the people in Paris know more clearly where they stand.

The British Government says that it welcomes the enlargement of Unesco's membership and the consequently increased attention being paid to the needs of developing countries, but that it has "felt unease" about the political aspects of certain programmes and that "above all" it fears that Unesco's programmes do not provide value for money. The letter goes on to say that the British Government will decide before the year is out whether to withdraw, looking for "significant indications of change" before then. The letter is accompanied by a list of what the British Government thinks should be done both to concentrate Unesco's programme on what it does well — or passably well — and to spend its resources more fruitfully.

Style apart, neither letter will be ignored. Unesco's willingness to open its books to the visiting audit from the United States (now in Paris) is one sign of that. The British letter, by being more specific, may be resented for appearing prescriptive but the points it makes are too cogent to be shuffled off, but are in any case backed up by the threat of a withdrawal. What neither letter says, however, is how Mr M'Bow should set about rescuing the agency of which he is the head from eventual collapse. Unesco is a potentially valuable organization, not merely because of the kinds of things it might do but because there are political circumstances (in India, or Yugoslavia, for example) where Unesco aid is more acceptable than bilateral aid. It is run down because it has been badly administered, because it pretends to be an expert body when it is not and, strangely, because it is desperately short of good ideas. If Mr M'Bow wants to save Unesco (or merely his present job), he should bend his energy to creating an imaginative role for an organization that has become dull. Or he should quit. □

Japanese budget

Science benefits despite cuts elsewhere

Tokyo

SCIENCE, particularly basic science, made a few small gains even from Japan's most stringent budget for almost thirty years.

After a long battle against the opposition parties in the Diet, with the April budget deadline looming ever closer, the government has finally won agreement for a 0.5 per cent cut in public expenditure during fiscal year 1984-85. Even so, the government will still be short of cash, and will have to issue bonds worth around $Y2 \times 10^{13}$ ($\$8 \times 10^{10}$) to cover expenditure, adding to the $Y10^{14}$ of public debt already incurred.

Expenditure increases have been granted in only a few areas: defence (plus 5-6 per cent), overseas development aid (plus 6 per cent) and science and technology (plus 0.7 per cent). None of the big three science and technology bodies shown in the table — the Science and Technology Agency (STA), the Ministry of Education, Culture and Science (MESC) and the Ministry of International Trade and Industry (MITI) — has gained much in total, but all have managed to protect their basic research programmes.

The biggest increase of all is for the new projects in areas related to computers and the life sciences. MITI's fifth generation computer project, the plan to build an "intelligent" computer which has sent Western governments scrambling to support artificial intelligence research, receives an 88 per cent budget increase. Close behind comes a 43 per cent increase, within MITI's large-scale industrial technologies programme, for research towards building the world's most powerful supercomputer. The goal is a machine capable of 10,000 million floating point operations per second, with applications in areas such as weather forecasting and the design of nuclear warheads.

All ministries, including the Ministry of Agriculture, Forestry and Fisheries and the Ministry of Health and Welfare (MHW), have increased expenditure in the life sciences, concentrating on biotechnology and cancer research. Precise budget increases are hard to calculate, for Prime Minister Nakasone's approval in June last year of a comprehensive "ten-year strategy for cancer control" has led to a recategorization of some funds for life science research.

There is little doubt, however, that total expenditure on fundamental research into molecular biology and the treatment of cancer is growing now that each ministry has established special "anticancer" funds (MHW $Y1,600$ million, MESC $Y1,200$ million, STA $Y7,700$ million, STA Radiological Institute $Y400$ million) as well as supporting cancer-related research in their general budgets. Research is being

coordinated under seven main themes: oncogenesis, carcinogenesis, early diagnosis, chemotherapy, radiotherapy and immuno-modulation and the use of monoclonal antibodies.

The budgets of the various agencies of the Japanese Government are as follows:

Science and Technology Agency. The budget for "special promotion funds", the interministerial grants for basic research thought vital to Japan's long-term prosperity which are given out directly by the prime minister's own advisory council, is further increased. Priorities are now recombinant DNA research, the development of new materials and cryogenic tech-

Japanese science budget

Science and Technology Agency	Y (thousand million)	Percentage increase or decrease
Total science-related budget	408.1	+ 2.4
Special promotion funds	6.4	+ 2.4
ERATO	2.3	+ 3.9
Nuclear energy	210.2	- 0.7
NASDA	85.9	
Cancer campaign	7.7	
RIKEN	9.6	+ 13.0
Disaster prevention	2.1	- 5.3
Expo '85	26.7	+ 4.6
Ministry of Education, Culture and Science		
Total science-related budget	282.9	+ 0.2
Grants-in-aid	40.5	+ 2.5
Research institute grants	100.4	+ 2.0
Special grants for large-scale facilities	22.7	+ 13.0
Ministry of International Trade and Industry		
Total science-related budget	232.7	+ 3.4
Basic technologies for future industries	6.0	+ 1.7
Large-scale industrial technologies	11.1	- 30.0
Sunshine project	39.8	- 5.2
Moonlight project	9.6	+ 1.0
Fifth-generation computer project	5.1	+ 88.0

nologies. But there is also an increase for Project ERATO which, by its support for work by small interdisciplinary groups on novel and sometimes eccentric themes, sets out deliberately to stimulate creativity.

The budget for research at STA's two massive nuclear energy institutes, the Power Reactor and Nuclear Fuel Development Corporation and the Japan Atomic Energy Research Institute, is slightly down but the figures conceal a large increase (33 per cent) for Project Monju — the building of a prototype 300 MW fast-breeder reactor by the late 1980s.

Expenditure on the commercial space programme by the National Space

Development Agency (NASDA) remains static although big increases are likely next year as the plan to build the H2 rocket, capable of putting 200-kg satellites into orbit, gets under way (see *Nature* 308, 3; 1984).

For the second year running, a big chunk of STA's budget is swallowed by preparations for Expo '85, the international exposition on the theme of "Science and Technology for Man at Home" which will open in Tsukuba Science City in March next year. Expenditure on Expo will practically cease in the next fiscal year and various groups are already eyeing the spare cash that will then become available.

Of the research corporations attached to STA, the Institute of Physical and Chemical Sciences (RIKEN) has the largest budget increase. Part of this represents continuing investment in the construction of a heavy ion accelerator.

Ministry of Education, Culture and Science. Grants to research institutes and grants in aid of research both increase a little but there is a bigger increase in "special funds" for large-scale facilities. Much of the extra will go to the High-Energy Physics Laboratory (KEK) for the 30-GeV per beam electron-positron collider (TRISTAN) at Tsukuba Science City. The project is on target, with the first results still expected in autumn 1986.

Also blessed among MESC's research laboratories are the National Institute of Genetics, which acquires the coveted status of a National Research Institute for Joint Use by Universities (see *Nature* 305, 372; 1981); the Institute for Space and Aeronautic Science which receives a 10-15 per cent budget increase, mostly earmarked for the launch of the Halley's comet probe, Planet-A; the University of Tokyo's Astronomical Observatory, which gains further investment in the Nobeyama millimetre-wave telescope; Kyoto University's Institute for Mathematical Sciences, where a new section of algebraic analysis is to be set up; and Osaka University's Microbiological Institute, which adds a section to study the molecular biology of cancer.

Ministry of International Trade and Industry. The ministry's "Basic Technologies for Future Industries" programme, made up of long-term research projects on biotechnology, new materials and new electronic devices, continues to be fully supported, giving credence to the avowed aim not to cut back on fundamental research even when funds are tight. Some of the more conventional programmes, the Sunlight programme for the development of new energy sources and the Moonlight programme for energy conservation, have done less well. Within the Large-Scale Industrial Technologies programme, which covers nine projects ranging from robot flexible manufacturing systems to computerized sewing systems, only the supercomputer project and the "robots for extreme environments" projects will see budget increases.

Alun Anderson

Anti-satellite weapons

Reagan backs tests in space

Washington

PRESIDENT Reagan has decided against renewing talks with the Soviet Union on a treaty to ban weapons for destroying satellites in space. In a report to Congress last week, he said he could not foresee a new arms control treaty for space that would serve the interests of the United States and its allies. And he made it plain that the administration wants to press ahead with tests in space of a controversial new anti-satellite (ASAT) weapon to deter the Soviet Union from using its own more primitive system.

But the president announced last week that the United States would submit to the UN Committee on Disarmament a draft treaty to ban the production, possession and use of chemical weapons.

The president issued the report on satellite weapons in order to release \$19 million for procurement of the weapon, a homing interceptor launched from beneath the wing of an F-15 fighter (see below). Congress voted the money last year, but put it aside pending a report on plans for negotiating a treaty with the Soviet Union to ban or limit existing and future ASAT systems. Weapons of mass destruction in space are prohibited by the outer space treaty of 1967, which does not, however, constrain the development of weapons designed to destroy satellites.

Technically, publication of the report leaves the administration free to spend the money, even though it has decided against formal negotiations for the time being. The US Air Force has already tested the new weapon within the atmosphere, but tests against targets in space are prohibited by the terms of a Senate stipulation last year that the president must first certify that he is trying "in good faith" to negotiate a ban and that tests are vital to national security.

Last week's report is nevertheless full of arguments against a comprehensive ASAT ban. It says it would be hard precisely to define anti-satellite weapons and virtually impossible to prevent cheating. It says the United States may need an ASAT system in war to shoot down Soviet ocean reconnaissance satellites, and that it would be "destabilizing" to leave the Soviet Union in possession of the only operational ASAT; should the Soviet Union shoot down a US satellite during a crisis, the United States, unable to respond in kind, might be forced to escalate instead.

The report has disappointed many in Congress who fear that deployment of the US ASAT will pose such severe verification problems that a future ban on such weapons will be impossible to negotiate.

In 1981 and 1983, the Soviet Union submitted draft treaties to the United Nations banning the use of force in space, and has announced a unilateral moratorium on the launching of any kind of ASAT weapon.

These initiatives are dismissed by the administration as an effort to prevent development of the US weapon. The report claims that Soviet ASAT capabilities include an operational orbital interceptor system, ground-based test lasers with "probable" ASAT potential, the ability to disrupt satellite transmissions electronically and, possibly, to destroy satellites using nuclear armed Galosh anti-ballistic missile interceptors.

Recognizing the strength of congressional sentiment against an arms race in space, the report says the United States is prepared to discuss "a broad range" of space arms control issues in the Committee on Disarmament in Geneva. The administration might also consider negotiating something less comprehensive, such as a ban on weapons that could reach the high altitude satellites that provide early warning of nuclear attack. That may not be enough for Congress, however. Senator Paul Tsongas (Democrat, Massachusetts) is to force an early debate of a resolution calling for a general ban on weapons in space and on weapons designed to destroy objects in space.

Peter David

Soviet and US approaches to ASAT

THE Soviet ASAT weapon is an explosive satellite placed in a low-Earth orbit by a modified version of the SS-9 intercontinental ballistic missile. When the satellite approaches its target a small explosive device is detonated, destroying the target with shrapnel.

The US weapon, not yet tested against a target in space, consists of a non-explosive miniature homing vehicle (MHV) designed to destroy its target by impact. The MHV is launched by a small two-stage rocket fired from beneath the wing of a modified F-15 aircraft. An inertial guidance system guides the device to the appropriate location in space, after which the MHV separates from the rocket and closes on the target by means of infrared sensors.

Critics of the US programme complain that, unlike its unwieldy Soviet counterpart, the compact weapon would confound attempts to verify a treaty banning ASATs. The Soviet weapon, by contrast, would have to be launched from a limited number of launch-pads by large and easily-identified boosters. The Soviet system is believed to have been tested 20 times, most recently in 1982. Six tests of a later version of the weapon, designed to home in on heat emitted by its target, are all believed to have failed.

The Reagan Administration argues that the Soviet weapon poses verification problems of its own. Although the SS-9 is large, the satellite interceptor is small. A covert supply could be maintained even if the

Research secrecy

Universities in scrap with DoD

Washington

RELATIONS between US research universities and the Department of Defense (DoD) are continuing to deteriorate. Cornell University has turned down a \$450,000 electrical engineering research contract from the US Air Force because it would have imposed an unacceptable degree of secrecy. Meanwhile, the presidents of Massachusetts Institute of Technology, the California Institute of Technology and Stanford University have confirmed that they have written to DoD and to the White House science office saying they cannot accept the publication restrictions proposed in a new DoD directive, DODD 2040.2 (see *Nature* 29 March, p.389).

Cornell's decision came after months of negotiation with the project sponsor, the Wright Patterson Air Force Base in Ohio. Dr Robert Barker, the university's vice-president for research, said the Air Force withdrew the contract when Cornell refused to promise to seek prior permission from the Air Force before any technical

Soviet Union claimed that it had dismantled existing weapons. And it would not take long for the Soviet Union to divert missiles from other tasks to launch the interceptors into orbit. Another problem is that many space technologies not specifically designed to destroy satellites could do so.

"Gee, Constantin—why didn't we think of this before?"



There is no shortage of targets for ASAT weapons. The Soviet Union launches more than 100 civil and military satellites a year, about five times as many as the United States. But US satellites last about five times as long, leaving each side with about 100 active military or military-related satellites in orbit at any time. But the most critical satellites, those that would be used for early warning and control in a nuclear war, tend to be in geosynchronous orbit beyond the reach of the present generation of ASATs. The maximum altitude of both the US and Soviet ASATs is believed to be less than 1,500 miles.

Peter David

data generated by the three-year project were communicated to foreign nationals. Cornell had already agreed to let the Air Force vet foreign nationals recruited directly for the project. But the university said that since "secret research" was banned at Cornell, it would be impossible to keep foreign nationals out of the project laboratory or to curtail normal scientific conversation within the department of electrical engineering. Last year, thirteen foreign scientists were employed in the department.

The universities hope the Cornell incident and the letter from university presidents will convince DoD that universities are in earnest when they say they would sooner drop defence department contracts than accept an abridgement of their right to publish. When university spokesmen first raised the threat, at a meeting last month of a sub-panel of the DoD-University Forum, Dr Edith Martin, the department's Under-Secretary for Research, implied that they were bluffing.

Although her comment could sour the atmosphere at the next full meeting of the forum later this month, the universities are anxious to find some form of compromise with DoD. After the collapse of the Cornell contract, for example, Dr Barker travelled to Washington for extensive talks with Dr Leo Young, director of research and laboratory management at the Pentagon. He explained that Cornell and other universities had difficulty in understanding how DoD's category of "sensitive" research differed from classified research — which many universities refuse to undertake.

In a related issue, the research community has responded critically to new draft regulations setting out the circumstances in which the Secretary of Defense can refuse to divulge technical data requested under the Freedom of Information Act. The draft regulations, contained in a DoD directive, DODD 5400.XX, would allow DoD to withhold from the public technical data with military or space applications that could not be exported outside the United States without approval under the Export Administration Act or the Arms Export Control Act.

The regulations have been sharply criticized by the National Science Foundation (NSF), the American Association for the Advancement of Science (AAAS) and the Association of American Universities (AAU). In a letter to DoD, Mr William Carey, executive officer of AAAS, says that by setting out controls for unclassified technical data, the regulations appear to prohibit normal professional communication between scientists working on DoD-sponsored projects and their colleagues in other fields. Dr Robert Rosensweig, president of AAU, has asked DoD to exclude university research from the rules altogether.

Peter David

UK civil service science

Careers congeal on cuts

CAREER prospects for the mainstream scientists in the British Civil Service have been seriously affected by manpower restrictions, according to a draft study carried out by the Institution of Professional Civil Servants. Although young "high fliers" are still being recognized and given rapid promotion, most scientists in all grades now face a "substantially longer" wait for promotion than in 1978.

In the past five years, there has been an overall reduction of 11 per cent in the levels of staffing for the main grades and of more than 20 per cent in junior grades. The study, produced by a working group examining the effects of the cuts, concludes that the reductions of up to 75 per cent in the recruitment of lower grade staff will create serious management problems in the future. But the rate of loss from the service has also decreased, so the rate of promotion on the typical route through the civil service grades has markedly declined. Things may not be so bad for all young graduates recruited into the service, however, some of whom can still expect to become Senior Principal Scientific Officers by the age of 33, with a salary of £15,600–£20,800 per year.

Mr Bill Brett, assistant general secretary of the institution, says the fundamental

problems of manpower structure will damage both the sciences and the civil service. Many scientists employed by the research councils are on a similar grade system and have been similarly affected by restrictions on manpower and, consequently, on recruitment.

The figures analysed in the latest study may conceal large differences between government departments, an issue the working party sought to investigate. Attempts to do so "have been thwarted by the Treasury's refusal to provide departmental information".

The institution represents 15,500 scientific civil servants and 9,000 scientists employed by the research councils. It has been active in opposing the planned closure of the Letcombe Laboratory in Oxfordshire as part of the current reorganization of the Agricultural and Food Research Council. According to Mr Brett, promotion prospects for those that stay in the council are certain to be badly affected, and about 600 jobs are now expected to be lost. The institution will now be considering a major campaign designed to influence the annual forward planning exercise carried out by the research councils and used as a basis for settling the share-out of the science budget.

Tim Beardsley

Plant conservation

Rescue plans afoot

TEN per cent of the world's flora — 25,000 of 250,000 taxa — are in danger of extinction, according to the World Wildlife Fund and the International Union for the Conservation of Nature and Natural Resources (IUCN). Under the chairmanship of the Duke of Edinburgh, the two organizations last month launched a campaign to encourage worldwide plant conservation.

Funds raised by the World Wildlife Fund will be allocated on the basis of a list of priorities drawn up by IUCN. Apart from a range of *in situ* conservation activities, for example in the Pleistocene refuge areas of the Cameroon forests, at Korup in India and on the Andaman and Nicobar Islands, the campaign's aim is to create a framework for concerted action and to involve other national and international organizations.

Information is central to this plan. The IUCN conservation monitoring centre at the Royal Botanical Gardens at Kew in London, which already holds details of 13,000–14,000 species of endangered plants, has also set up an additional database (SEPASAT), with help from the overseas charity Oxfam, to store information on economically useful plants from arid areas. There is talk of incorporating data held at the International Board for Plant Genetic Resources (see *Nature* 8

March, p.105) into the computer at Kew.

Having surveyed the activities of international organizations as they affect plant conservation, IUCN hopes to involve as many as possible in *in situ* conservation, for example the World Health Organization for medicinal plants. It will also promote *ex situ* conservation by widening the scope of the Botanic Gardens Conservation Co-ordinating Body. A micro-propagation unit at Kew is successfully rescuing casualties, in particular orchids and ferns. There is still a need for worldwide collaboration to monitor and coordinate garden collections.

Because perhaps 40 per cent of the world's pharmaceuticals come from wild plant sources, the Food and Agricultural Organization has been pooling knowledge on the medicinal properties of trees in selected developing countries. The United Nations Industrial Development Organization is considering placing small plant-based pharmaceutical industries in developing countries.

A major problem of conservation is law enforcement, and an investigation is being carried out of the international trade in threatened plants and to assess the ability of enforcement agencies in different countries to implement plant trade controls.

Sarah Tooze

British universities

Protests over proposed changes

BRITISH universities last week attacked government policy on higher education in the strongest terms they have yet dared to use. The Committee of Vice-Chancellors and Principals (CVCP) accuses the Department of Education and Science of "gravely underestimating" future demand for university education, while the government "is as a matter of deliberate policy providing for fewer places than would be reasonable even on the basis of its own projections". The committee dismisses the government's declared wish for a further shift toward scientific and technological subjects as representing "a view of society which we cannot accept".

CVCP's comments are made in its published response to the questionnaire circu-

lated to universities in November by the University Grants Committee (UGC). The questionnaire asked a wide-ranging series of questions on the possible future development of higher education. But several universities have rebelled and refused to answer a question which asks them to consider the likely consequences of an annual reduction of 1 or 2 per cent in the level of financial provision per student. The University of Aberdeen says bluntly "we cannot usefully be willing accessories before the fact to our own mutilation and we decline to give a detailed response to these destructive hypotheses".

The questionnaire was circulated in response to a request from the Secretary of State for Education and Science, Sir Keith

Joseph, for a "free and wide-ranging" debate. The vice-chancellors and principals are concerned that the debate has been undermined by the apparent removal of the prospect of level funding. Recently-published government expenditure plans appear to provide for cash increases that will be well below the rate of inflation. The universities protest that they are now having to turn away well-qualified candidates in far greater numbers than just a few years ago, while expenditure per student has fallen by about 10 per cent in real terms in the past four years.

CVCP believes that financial restrictions have hit research activity in the universities more badly than teaching. Many universities are making strenuous efforts to attract money from industrial companies for applied research, so academics are devoting more of their increasingly scarce time to securing outside sources of finance. But universities remain opposed to any erosion of their cherished right to spend their grant as they wish, although there are differences of opinion among the vice-chancellors.

Most are opposed to any extension of "earmarking" of components of recurrent grant for specific purposes. Several independent review bodies have suggested that by this means scientific research might be protected, but CVCP feels that only the universities themselves can provide the necessary flexibility of planning. Dr John Burnett, principal of the University of Edinburgh and a vice-chairman of the committee, says UGC "probably doesn't have any idea" how much individual universities are spending on research, and that no central body could cope with the administration of research in all of Britain's universities.

CVCP proposes that universities should in future make a much more detailed input to UGC's planning. Since the collapse a few years ago of the historical quinquennial system for allocating recurrent grants there has been, according to Dr Burnett, a lack of dialogue between universities and the grant-giving authority. By providing full information on how they would use their grant, Dr Burnett hopes, universities will be able to ensure a better distribution while retaining their independence.

Most universities continue to support the principle that they should be financed from two separate sources, UGC for general support and the research councils for specific research. And they maintain that there is sufficient diversity within the system to cater for all the demands imposed upon it. Not all universities support the continued existence of UGC, however. The University of Salford, which has earned much of its income from external consultancies since its grant support was dramatically reduced a few years ago, accuses UGC of being "intellectually partial, managerially weak and administratively overstretched". **Tim Beardsley**

NIH grants

Indirect costs under attack again

Washington

INDIRECT costs continue to eat up an increasing share of the research budget at the National Institutes of Health (NIH). And according to a report from the General Accounting Office (GAO), the chief reason is the failure of NIH to audit the indirect cost rates claimed by universities receiving NIH grants.

Indirect costs, a euphemism for overheads, cover administration, depreciation on buildings, services such as libraries and other expenses incurred by the universities as a result of taking on federal research projects. Since 1966, when Congress lifted a restriction that had held indirect cost reimbursements to 20 per cent of direct research costs, the proportion of NIH money going to indirect costs has steadily climbed, reaching 43 per cent of direct costs in 1983. Some institutions have much higher rates; Harvard University, for example, charges indirect costs at 70 per cent of direct costs.

According to GAO, from 1978 to 1983 NIH audited the indirect cost claims of only 47 of the 700 institutions receiving grants. Significantly, 40 of those audits (the others contained insufficient details to draw any conclusions) disallowed a total of \$57.8 million in indirect costs. The unstated implication is that NIH could save hundreds of millions of dollars each year by closer scrutiny of the universities' claims. GAO says that many requests for audits by the government's contract negotiators went unanswered.

NIH officials are said to blame rising indirect costs on the universities' adoption of "sophisticated cost allocation techniques which maximize the amount of allowable reimbursements", but GAO finds that government negotiators in at least some cases allowed huge increases in indirect costs to pass unquestioned. Although

negotiation with the universities in general brought about a reduction in the universities' claimed indirect costs, GAO investigators could find no written explanation for the many large increases of the overload rate allowed.

Departmental administration costs have been the hardest to control, as they are based on largely subjective "effort reporting" by faculty members — a declaration of time spent on research (direct costs), administration (indirect costs) and teaching (not reimbursable). Effort reporting has been decidedly unpopular with faculty members, who have complained that it is impossible to categorize their functions and that the exercise thus becomes meaningless. GAO agrees, but its report also notes that the government has virtually no way to verify the universities' claims for departmental costs, which account for 35 per cent of total indirect costs claimed by large universities.

The GAO report is at least the twelfth in as many years to complain about rising indirect costs. None has yet had the least effect. An attempt two years ago by the Reagan Administration to impose an across-the-board 10 per cent reduction in indirect costs was laughed out of Congress, which apparently remains reluctant to cut any part of the NIH budget.

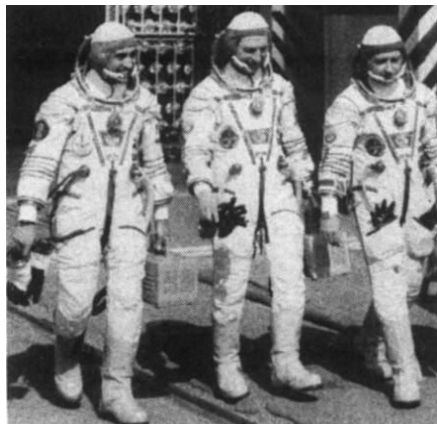
One GAO recommendation that may, however, carry some weight — and which would not require congressional approval — is that NIH should in advance set departmental administration costs as fixed allowances. As things are, small institutions (those receiving less than \$3 million in direct grants) can opt for such a fixed rate, set at 20 per cent of the salaries and expenses of deans and department heads. Last year, NIH negotiated a similar arrangement with Yale University.

Stephen Budiansky

Soviet-Indian space mission

Going along for the ride

THE visit of Squadron-Leader Rakesh Sharma to the Salyut-7 space station has been widely acclaimed in the Soviet media as marking a new phase in the programme of "international" flights; for the first time the visitor came from a non-aligned country, India. (Apart from the Frenchman Jean-Loup Chrétien, all guest cosmonauts have hitherto come from the Comecon bloc.) Public reaction in India has been predictably euphoric. The



Indian cosmonaut Rakesh Sharma (left) joins Soviet cosmonauts Yuri Malyshev (centre) and Gennady Strekalov.

political importance of the event was highlighted by Sharma's conversation from space with the Indian Prime Minister, Mrs Indira Gandhi, in which he skilfully quoted the national poet Iqbal to imply that, from space, India looks "the best in all the world".

In the long term, however, the relevance of the mission to India's own space programme is problematic. For India's space effort rests firmly on the two "legs" of remote sensing and telecommunications, and aims at eliminating its dependence on foreign hardware as rapidly as possible. Media claims that the flight proves that India is a "technologically advanced" nation seem somewhat illogical. The launch of the indigenous Rohini satellite or the SRV launch vehicle can rightly be cited to substantiate such a claim. Guest cosmonauts to Salyut stations in the past, however, have included representatives of Mongolia, Vietnam and Cuba, which cannot yet claim an "advanced" level of technology.

The mission's greatest relevance to the routine Indian space programme lay in the "Terra" high resolution photographic survey of the Indian subcontinent, including the Andaman and Nicobar Islands (where it was hoped to find indications of oil, gas and mineral deposits) and of remote areas of the Himalayas and Karakoram.

The materials science experiments, on the other hand, although said to be "dictated by the needs of the Indian economy", centred on a standard feature

of previous joint missions, the production of alloys difficult or impossible to obtain under normal gravity.

The most "Indian" note, from the Soviet point of view, was provided by the medical programme, which included yoga exercises performed by Sharma and which, it was hoped, might provide a means of alleviating the adverse effects of weightlessness on the locomotor system. (Moscow Radio was careful to point out that Sharma is not a yogi but an "ordinary person", since Soviet ideology tends to be

wary of the philosophical aspects of yoga.) India also provided a portable "vector cardiograph" for monitoring the bio-electric activity of the heart at rest and under gradually increasing physical loads. The Indian medical team also resumed a line of research studied by the joint Polish-Soviet mission of June 1978: the physiological and psychological changes in conditions of weightlessness. Since India has no regular space medicine programme, and since no country has so far been invited to send a second cosmonaut to the Salyut station, the results of these experiments, although helpful to spacefarers in general, are unlikely to benefit India directly.

Vera Rich

Biotechnology

Low risks at a premium

THE Sun Alliance insurance group and Reed Stenhouse UK Ltd are planning to launch "Biotech Protection" — claimed to be the first insurance package geared specifically to the needs of biotechnology laboratories. The policy includes all-risks cover on assets and earnings against cross-infection of cultures, contamination of premises and seizure or closure of premises by safety authorities. Legal liabilities to third parties for environmental pollution and innocent or fraudulent breach of confidential information can also be insured.

According to Reed Stenhouse, market research has shown that many biotechnology laboratories are not adequately insured against the risks peculiar to their work. Celltech Ltd, the UK biotechnology company, refuses even to discuss its insurance protection. Others in the industry are surprised that seizure by civil authorities is

an insurable risk. And there is some scepticism about whether the risk of breach of confidential information could be assessed or a claim proved.

Mr Ken Davis of Reed Stenhouse is undeterred by such questions. His company will provide full advice to its clients on safety and security issues, he says, and the company's "loss engineers" will be able to assess the value of cell lines and evaluate dangerous practices. He denies that cover for disclosure of confidential information will encourage greater secrecy, because most companies already have good arrangements with their staff on such matters. But if the loss engineers were dissatisfied then they would be able to make appropriate recommendations. The package will be launched at the Biotech Europe exhibition, to be held in London during May.

Tim Beardsley

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 30 Mar.	Change
14	10½	Biogen (Switzerland)	13	13	0
2	1⅜	Bio-Logicals (Canada)	1½	1⅜	+⅛
14⅞	10⅞	Bio-Response (USA)	10¾	11	+¼
14	10½	Cetus (USA)	11¼	12⅞	+⅞
10⅞	6⅛	Collaborative Research (USA)	7⅝	8⅞	+½
19⅞	14¾	Damon (USA)	17½	18⅞	+⅝
26¼	13	Enzo-Biochem (USA)	16¾	17½	+¾
10⅞	6¾	Flow General (USA)	7¾	7⅞	-⅜
42¼	31¼	Genentech (USA)	33½	36	+3½
10¾	6	Genetic Systems (USA)	6⅞	6½	-⅜
17¼	12⅞	Genex (USA)	13	13½	+½
23	12¼	Hybritech (USA)	14¾	13½	-1¼
16¼	11½	Molecular Genetics (USA)	13¼	14	+¾
15½	10¼	Monoclonal Antibodies (USA)	11	11¼	+¼
60⅞	45½	Novo Industri A/S (Denmark)	52¼	48⅞	-3⅝
22¾	16½	Pharmacia (Sweden)	16¾	18⅞	+2⅞

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 169 on 30 March, compared with 163 a month earlier. Data from E.F. Hutton, Inc.

Artful scientists

SIR — In 1878, J.H. van't Hoff (who became the first Nobel laureate in chemistry in 1901) read an inaugural lecture at the University of Amsterdam entitled "Imagination in Science". In that lecture, van't Hoff suggested a correlation between scientific eminence and creativity in the fine arts and literature based upon a study of more than 200 scientific biographies. I am attempting to verify van't Hoff's thesis with regard to important scientists since 1800. Van't Hoff himself was a poet, as were his colleagues Walther Nernst, Fritz Haber and Richard Willstätter. One can add to the list Ronald Ross, Julian Huxley, E.N. da C. Andrade and Jacob Bronowski. van't Hoff's friend Wilhelm Ostwald was a painter, as were Louis Pasteur, F.F. Runge, Ogden Rood, Ernst Haeckel, Theodor Boveri, Frederick Banting, Lord Adrian and C.G. Jung. C.H. Waddington wrote a history of twentieth century science-art interactions. Ernst Mach, Hermann von Helmholtz, James Jeans, Max Planck, Albert Einstein, Woldemar Voigt and many others had an intense interest in music. J.B.S. Haldane, C.P. Snow and Leo Szilard wrote works of fiction.

The problem is that all too often these nonscientific activities are not mentioned in obituaries or standard biographies since they are regarded as unimportant. The actual products (paintings, sculptures, manuscripts and so on) are even more difficult to locate. Yet to those interested in nonverbal thinking and scientific creativity, van't Hoff's hypothesis is an intriguing one that can only be studied in the light of the relevant non-scientific products of the scientist's imagination. Further references to scientists of significant professional stature who were notably creative in other fields would be appreciated, as would descriptions of collections or sources of reproductions of creative artefacts.

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Proxy records

SIR — The article "Frost rings in trees as records of major volcanic eruptions" by LaMarche and Hirschboeck (*Nature* 307, 121-126; 1984) included the phrase "Recent development of proxy records of past eruptions . . ." But what does this mean?

This use of "proxy" is unknown to the *Oxford English Dictionary*, and two US colleagues I have consulted cannot tell me the precise meaning of the phrase "proxy records".

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A Homeric riddle solved . . . again

white + red + black + grue + yellow + yellow + grue + green + blue + purple and pink and orange + brown + orange

SIR — I note with interest the recently preferred explanations of Homer's "wine-dark sea". The field of anthropological linguistics provides another, more elegant solution.

All languages possess between two and eleven basic colour terms, that is, colours that cannot be described as "shades" of other colours¹. They enter a language in a fixed order², as shown above, where "grue" covers both the green and blue areas of the spectrum. "Grey" can enter at any time after the first five.

As English has all eleven basic colour terms, we find it difficult to cope with

systems such as Homeric Greek, which divides the spectrum into only four parts, the equivalents of "white", "black", "red" and "yellow"³, and the related adjectives cover a correspondingly wide spectral band. (Compare Old Irish *fion dubh* "black wine" and *glas-muir grue sea* "within a five-colour system.")

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1. Berlin, B. & Kay, P. *Basic Color Terms* (University of California Press, Berkeley, 1969).
2. Kay, P. *Language* 4, 257-270 (1975).
3. Capell, A. *Studies in Sociolinguistics* (Mouton, The Hague, 1966).

SIR — Homer's seas were not always "wine-dark". Often, they were "gray" (*Iliad* 14:190, *Odyssey* 2:261. . .) or, "bright", "black", even "violet" (*Od.* 5:57, 11:107). Surprisingly, oxen could be "wine-dark" too (*Il.* 13:703), while ships might have "purple-red" cheeks (*Od.* 11:124, 23:271. . .). Cloaks worn by nobility were invariably plain "purple" (*Od.* 4:154). Yarn and wool, however, might be "sea-purple" (*Od.* 6:53, 6:306) or "violet-dark" (*Od.* 4:135). Sometimes a "steel" or, "bright blue" was mentioned (*Il.* 18:564, *Od.* 7:87) or a "grayish blue green" — glaucous.

The mysterious, often-used epithet "wine-dark" very possibly did come, in part, from the bluish-purple colour of wine mixed with water (*Od.* 7:164. . .) — as has been suggested by others.

Mostly, however, it was probably a discreet salute to the surly, dark-haired sea-god, Poseidon, ever raging against Odysseus to take his life. "Flaming" or "swarthy" wine, αἶθρα (*Il.* 1:462, 4:259 . . .) was used for libations to the gods. Poseidon spent his leisure moments feasting with the Ethiopians, Αἰθρα, "who dwell sundered in twain, the farthermost of men, some where Hyperion sets and some where he rises". His watery realm divided Zeus's heaven and Hades' underworld (*Il.* 15:189). He was the jealous husband to Amphitrite, the ancient sea goddess and his vengeful roiling of the sea with quakes, storms and winds was akin to a besotted spirit's. His name, Ποσειδάων surely derived from ποῖος, (1) "drink", (2) "husband" and, δαῖω, (1) "set ablaze", (2) "divide" (as with the trident).

Like Odysseus, Homer the poet-minstrel was probably a frequent sailor from kingdom to kingdom. He may also have felt assailed by treacherous seas.

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SIR — It has been suggested^{1,2} that Homer addressed the sea as "wine-dark" either because his wine was blue or because dust clouds at dusk made his sea crimson.

The true reason is quite different but equally wonderful. Our colour system is primarily based on the frequency spectrum of light: not so the Homeric one. It was that great classical scholar and part-time Prime Minister, William Ewart Gladstone, who first noted that Homer used colour terms to refer primarily to distinctions of light and shade³.

Thus the word *khloros* describes both green leaves and yellow sand: meaning something like "pale". Whereas *glaukos* describes grey eyes and green willow leaves: meaning "glinting". So Homer's wine-dark sea merely had the albedo of wine, not its frequency.

Gladstone suggested "the organs of colour and its impression were but poorly developed among the Greeks of the heroic age" (cited in ref.4). But we need not assume there was anything physiologically the matter with the ancient Greeks. Indeed several tribes today have a notion of colour remarkably similar to that of Homer. For example⁵ the Jale of New Guinea have but two colour terms, corresponding to "dark" and "light"; and the Tiv of Nigeria have three colour terms, meaning "dark", "light" and "reddish".

One final speculation. According to tradition, Homer was blind. Perhaps he was just colour blind?

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1. Wright, R.H. & Cattley, R.E.D. *Nature* 303, 568 (1982).
2. Rutherford-Dyer, R. *Nature* 306, 110 (1983).
3. Gladstone, W.G. *Studies on Homer and the Homeric Age*, 457-499 (Oxford University Press, 1858).
4. Woodworth *Psych. Bull.* 7 325-334 (1910).
5. Berlin, B. & Kay *Basic Color Terms* (University of California Press, Berkeley, 1969).

Excellence in the midst of poverty

India has set ambitious goals for science and technology — self-reliance and the relief of poverty. Some great things have been accomplished, but much effort is frustrated.

INDIA, where zero was invented, often describes itself as the third nation in the world in science and technology. The claim is based on the output of graduates in science and technology from Indian universities, which is exceeded only in the United States and the Soviet Union. Naturally, the ranking would be different if the figures were adjusted for population size, but the Indian claim is justified qualitatively even by casual inspection. The newspapers are fuller than in the West of reports of technical developments, or of the speeches made at scientific meetings. (And government agencies seem always to be advertising technical vacancies.) The major cities have laboratories scattered in their inner suburbs. People are alert about the doings of the scientific enterprise, especially in the Antarctic or in space. And,

knowing how much importance is attached to science and technology by the Government of India, and how much is being spent on them, people — even technical people — are forever asking “Why aren’t we doing better?”

What follows is not a presumptuous answer to that question but a comment on it. First, it seems not fully to be appreciated in India that the question is commonly asked elsewhere, in places such as West Germany, the United Kingdom and even in the United States. Second, many in India overlook what has been accomplished. The most staggering achievement is the increase of agricultural production in the past two decades, which has allowed agricultural economists the luxury of worrying about the import bill for coconut oil and not to have to worry about the external source of

next year’s grain. While the most obvious transformation of farming has been in the rich irrigated areas such as the Punjab, the agricultural extension services which have been strengthened nationally are a powerful insurance for the future — a more durable asset than a handful of Nobel prizes. But none of this advance would have been possible without the small army of geneticists, plant physiologists, soil scientists and the like who have managed and who maintain the green revolution.

For the rest, disappointment must be a function of expectation. And it is true that many of the country’s best-known technical organizations have sprung from hopes that their creation would solve particular problems. The Council for Scientific and Industrial Research was to have made manufacturing and mining industries profitable, yet exports of engineered products are declining. Why have an Indian Council of Medical Research (and an All-India Institute of Medical Science) if diseases banished elsewhere are still common and population growth unchecked? Why does the Atomic Energy Commission’s goal of generating 10 per cent of the country’s electricity from uranium so steadily recede? What use is an Electronics Council (now the Department of Electronics) if the telephone system does not function properly? Could there even be a fallacy in the common assumption that science and technology are, for India, the keys to development?

Fortunately, the present government is not infected by such doubt. There are few technical communities elsewhere that enjoy the sense now common in India that circumstances have recently improved and are still improving. Who would have guessed five years ago that there would now be a permanent Indian base in the Antarctic? Or that the rudimentary experiments ten years ago with satellite-to-village broadcasting would so quickly have been followed by the prospect of a national system using indigenously-built satellites? In the support of academic science, the government has also moved decisively, with the result that good projects are not being frustrated for lack of funds. The government seems to be taking the empirical view that what has been spent has returned social, economic and intellectual benefits, and that spending a little more can only yield more of the same. That is a good first approximation to a strategy.

Beyond that, however, the sceptical

India’s inheritance of ambivalence

THE bias of this survey, written by John Maddox with contributions (as noted) from Vera Rich, is as follows:

Among developing nations, India has by far the best chance of succeeding. The doubt is not whether but when. The country’s greatest asset is not its natural resources (which are nevertheless vast) but the ingenuity and articulateness of its people.

The difficulties are well-known and formidable. The huge uncouned population (the registration of births and deaths is not everywhere compulsory or even practicable) is about 750 million and growing at 2.5 per cent a year. The percentage of illiteracy, officially estimated as a third, may be twice as much. Poverty is literal in the sense that large numbers of those who live in the estimated 700,000 villages have no regular source of cash of any kind. For them, to make a purchase is not possible.

India is culturally as well as geographically diverse. There are a dozen different regional languages, but the plan that Hindi should become the common language makes only slow progress. The state governments are constitutionally as independent of the Union government as are those of the United States but, because of regional political interests, often have a more powerful vested interest in resisting what “the centre” wishes. The Sikh rebellion in the Punjab during the past year is as worrying as the trouble in Northern Ireland.

In the circumstances, it is remarkable that so much has been achieved. The green revolution, however, did take place, with the result that India now exports some staple food. Urban prosperity has visibly increased — too fast, the purists say. Most large cities are awash with construction (but mercifully the authorities have halted the construction of tower blocks along the sea front in Bombay).

Two principles underlie what has been done — the conviction of everybody with an opinion on the subject that India, being a democracy, must remain a democracy and put up with the disadvantages thereof (notably bureaucracy and legitimate and litigiousness) and the conviction (going back to Nehru’s chairmanship of the Planning Commission before independence in 1947), that science and technology are indispensable to development. Considering the difficulties there have been, it is marvellous that these principles are still upheld.

British rule in India seems to have left both Indians and British with a curious ambivalence — both regret what might have been, some British tend to pointless reparation, many Indians make past colonialism a universal excuse.

These prejudices are not meant to show. But that the Indian telephone system is appalling, and a bizarre impediment to efficiency, is not a prejudice but a fact. □

Enthusiasm from the top

MRS Indira Gandhi is for the time being the heroine of Indian science. Since her return as Prime Minister in 1979, people in laboratories everywhere have been congratulating themselves that they again have a government that understands what laboratories are about. Mrs Gandhi is indefatigable in her sponsorship of science, as chairman of the governing bodies of organizations such as the Atomic Energy Commission and of the Council of Scientific and Industrial Research, of the Science and Technology Council, inaugural speaker at the annual Science Congress and an opener of countless exhibitions, laboratories and conferences.

How did this interest arise? "I've had it since I was a child", she says, explaining her passion for natural India. One of the first acts of her new government was to set up a Department of Environment (see p. 589). She has also appointed six or seven scientists as government secretaries (heads of departments) — the count is uncertain because the most influential of them all, Professor M. G. K. Menon, is now a member of the Planning Commission.

As is well known, Mrs Gandhi is a sharp and witty lady but when she speaks of "my scientists", one cannot tell whether she refers to her intimates or, maternally, to everyone.

So what does her government hope to do about the plight of the universities? A large part of the trouble, she says, is that "they're so terribly politicized". Some way has to be found of restoring authority to where it belongs. Those responsible must

take charge. She emphasizes how much is being spent on the universities, and that the country has hardly begun to tackle the problem of the education of the poor.

But Mrs Gandhi is also quick to insist that educational attainment is not a standard by which people should be judged. India's strength may be in the villages.

In these circumstances, why so much concern and resources for high technology, the space programme for example?



Because satellites are the only practical way of reaching all the poor. Then why not simply build the communications satellites, and let the Europeans, the United States or the Soviet Union bid to launch them? "We couldn't rely on that. Look what they did to us over the nuclear reactor contract!"

On the question (raised by the television

critic of *The Statesman* of Calcutta) whether it would be possible to deliver a working television set to every school when the supply of blackboards had been a failure, Mrs Gandhi scornfully dismisses "anybody who writes for *The Statesman*". And the question why the Indian telephone system does not function is turned with the remark that it too had been designed in the West.

The argument that if India is to succeed, it must find export markets by making some products superbly well, and better than other people make them, cuts very little ice. "The West", Mrs Gandhi says, "is basically a consumer-oriented society." India is different.

On the recurring problem of the poor, Mrs Gandhi says "You've no understanding how colonial rule impoverished India". But she considers that science and technology "could do a better job" in catering to the needs of the villages in simple ways such as the improvement of the tyres on bullock carts. She emphasizes again that India is a densely populated country and adds "my scientists tell me that we're still moving" and that India is getting smaller every year.

Mrs Gandhi's enthusiasm for science and technology, and her government's generous support of it, are most easily explained in strategic rather than economic terms. Self-reliance rather than self-sufficiency is the immediate objective but import substitution is also important for financial reasons. It also helps that people have some achievements to be proud of. "We are not feeling insecure", she says at one point. □

question needs more careful consideration. The range of scientific activity in India is vast. There are some hundreds of laboratories run by the government, its central agencies and the states, all of them essentially paid for by public funds. There are as many higher education institutes teaching science and technology. Some of these establishments are splendid by any standards (and some of these are described in the pages which follow). But elsewhere, quality varies from place to place. Both an academic and applied research, India's peculiar difficulty is that first-rate institutions are so few as to be a veneer that barely conceals the prevalence of the second-rate. Sometimes it seems that the practitioners have learned the rules of science — how to submit a paper to a journal — but not the content. This phenomenon is not much disputed. What matter are the causes and the cures.

The shortage of money is no longer a serious problem. The difficulties that were common as recently as ten years ago in purchasing new equipment seem to have melted away, at least at the better places. And while some people still work with microorganisms in conditions so unhygienic as to be a hazard for the experimenters, many laboratories are superbly

equipped. Travel funds, especially for going overseas, are still short, but the importance of keeping in touch with what is happening elsewhere is now more widely appreciated than ever, by laboratory directors, university department heads and by their ultimate sponsor, the government. There is no sign that this improving trend will be halted.

Sheer inefficiency is a more common problem. Some complaints are almost universal and persuasive. Young people have too little say in the design of the projects on which they work. The bureaucracy makes research harder and slower than it should be. Spending a small part of a grant already awarded may take days of negotiation with the institutional administrators, buying a simple piece of equipment may require that tenders should be invited from suppliers by advertisements in the local press. (Although people in India are fond of saying that the bureaucracy is the least welcome legacy of the British Raj, the practice of collecting useless information on multiple forms so badly printed that it cannot be digested seems an indigenous refinement.) The inefficiency of suppliers is a notorious cause of delay. Complaints such as these are not of course confined to India, but they are

nowhere more prevalent. Yet the experience of the better laboratories, many of them squarely in the public sector, shows that the bureaucracy is not indispensable and that research can be carried out on the collegiate basis shown to be effective elsewhere. There are three reasons why such self-inflicted impediments to achievement are not swept away: many people with responsibility for research were brought up in earlier more hierarchical times when responsibility equated directly with power over others, some use the system tyrannically to conceal their own weaknesses and in some places — the other side of the coin — the younger people cannot be trusted to function on their own. On the first two counts, the remedies are obvious, the third points at the deficiencies of higher education.

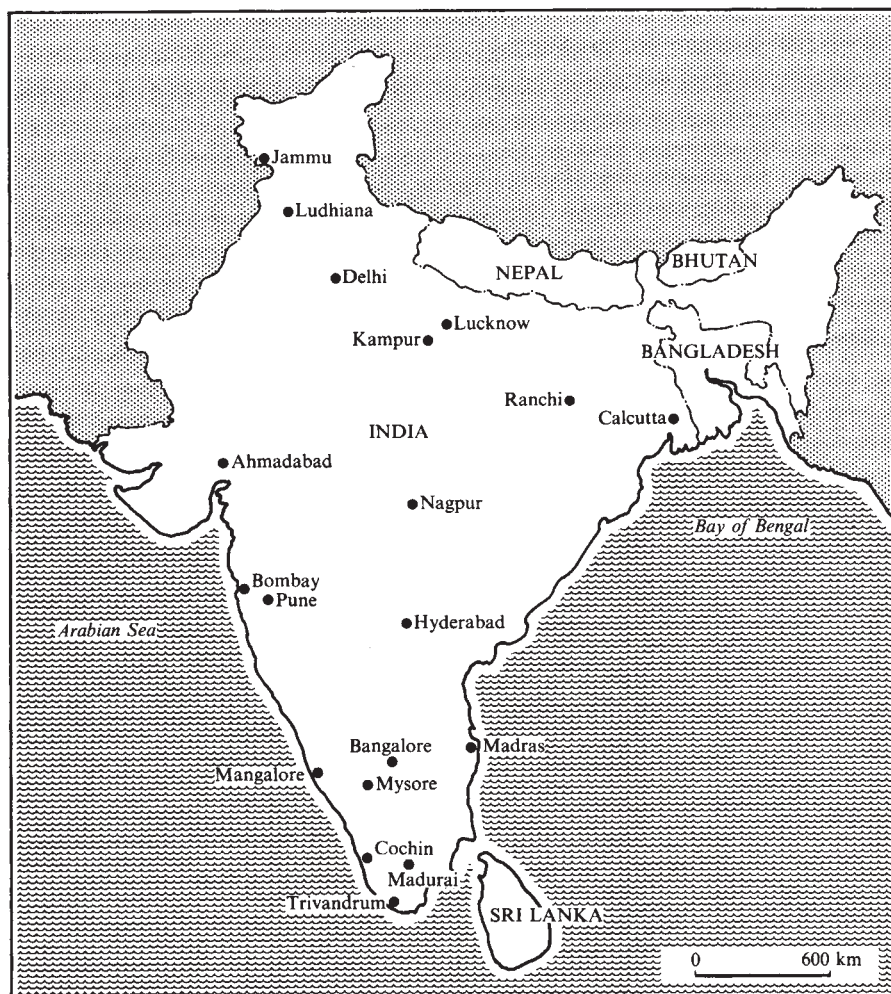
The conditions under which technical people are employed create other problems. Most work on the terms laid down for servants of the state or of central governments. While the salaries and allowances compare well with those of other public servants, the rigidity of the system of allowances (for housing, the cost of living and medical care) and of the promotion ladders is widely recognized to be an impediment to personal advancement and

mobility. Moreover, a person's appointment and promotion will be inordinately determined by paper qualifications and publications record. In circumstances in which the decisions of personnel committees can be challenged in the courts — one famous case has just been settled by the Supreme Court after 14 years (see p. 599) — it is natural for the authorities to shrink from using subjective criteria of promise and achievement.

Yet even the present system does not outlaw nepotism; when every graduate student is potentially a co-author, the head of a university department or a laboratory can further his friends' prospects by a judicious allocation of each year's student entry and in many other ways. While there is no hope of mass escape from public service, for employers on a sufficient scale do not yet exist, surely ingenious India can adjust housing allowances to encourage mobility and help give laboratory directors the courage of their convictions about people with the external review of internal appointments. As things are, the wonder is that so much has been accomplished in such stultifying circumstances.

Petty charlatanism is another scourge crying out for banishment. There is a lot of it about, especially in the journals. When publications are as important as in India, recycling old ideas in new papers is a constant temptation. The practice of senior authors' names being added to junior colleagues' papers is widespread (but sometimes junior people hope to improve the chances of publication by embellishing a paper with an important name). The refereeing system leaks intolerably, so that people fear giving honest negative opinions for fear of reprisals. The solution is clear: use referees from outside as well as inside India. The journals which say they cannot afford the postage should ask themselves whose interest is served by the present system. And in any case some journals (notably those of the Bangalore academy, the Indian Academy of Sciences) seem to manage. Out-and-out false claims are commonly suspected but rarely investigated seriously.

The influence of powerful men and institutions on the pattern of research is far too direct. In one of the largest technical communities in the world, it is surprising that the chairmen of advisory boards and the inaugural speakers at congresses should



be drawn from such a narrow circle. This is not to deny the value of experience of the system, but it is dangerous if a great man's chance remark can be converted overnight into a research programme that a mere laboratory director could mount only after battling for weeks with his administrators.

Letting wishes father the perception of reality is another common failing. The complaint last year by the parliamentary Public Accounts Committee that the Council for Scientific and Industrial Research had failed to produce colour television monitors in time for the Asian Games two years ago (see p. 586) is an illustration of the danger. Everybody knows that it is now possible to make integrated circuits that will drive television sets, but it does not follow that a suitable production line can be set up in an arbitrarily short time. The cultivation of realism about technological projects would be a great economy of time and temper. The government's enthusiasm for biotechnology may be a larger error of the same kind; the hoop-la on the New York stock exchanges seems to have blinded people in India to the likelihood that the economic pay-off will be slow to come.

The twin government policies of import substitution and self-reliance encourage a more general sense of unrealism in applied research. Behind the cloak of import substitution, even the practitioners are

hard-pressed to know the difference between original and derivative developments. Adapting technology developed elsewhere to Indian needs is never child's play, but can it be in India's best interests that so much bright enthusiasm should just now be invested in the replication in Indian laboratories of machines and communications networks using micro-processors that can be bought across the counters of retail shops elsewhere in the world? In due course, no doubt, these same machines will be manufactured in India more cheaply than they could have been bought, and self-reliance ("we can do it at a pinch") will have been satisfied as well. But might there not be even greater benefits in manufacturing some product that nobody else can make?

If private industry were more vigorous, questions like these would more easily be answered. But then, the administration of science and technology would also be simplified — government and the applied research laboratories would no longer have to second-guess the course of industrial development. Then, it would also be possible to hope for some flexibility in the pattern of research institutions with which India has been lumbered since independence. Even more obviously than elsewhere, it is much easier to create new organizations than to close them down or to carry through radical reform. Laudable

Conversions

THE unit of currency in India is the rupee, written here as Rs1 but also in India as /- , a reminder that during British rule the value of the rupee was that of the British shilling. Now, Rs15 are very roughly £1 and Rs10 are \$1 (US).

Counting is more difficult. The major multiples of 10 in the decimal system are 1,000, 100,000 (one lakh), 10,000,000 (one crore) and so on in multiples of 100. □

democracy is the restraint.

This applies more directly and more urgently to the universities of India (see p. 591). The University Grants Commission has been diligently devising incentives for universities to improve themselves, but is now faced with a stark choice. Either the system that it loosely controls must be accepted for what it is, a collection of diverse institutions which provide students with an education that varies in quality from place to place, in which case the commission will have to use its influence to combat the Indian legend that all degrees are equally valuable, or it will have to use its powers to strike some institutions off its lists. The first course is preferable because diversity itself is valuable. Either way, the political difficulties will be formidable.

The problem of the universities is important (see p.591 *et seq.*) not merely because universities everywhere are important but because the science-based success for which India has been striving since independence seems so constantly just within reach. Some group of industries may one day find itself overwhelmed by the demand for its products. The universities, now reviled for having too many students, will then be reviled for producing too few graduates in the field concerned. The justifiable complaint, however, is the variability of the technical competence of the academically qualified. The haunting question is not which opportunities may be lost in future but how many have been lost already for lack of people to carry through to fruition the bright ideas which abound.

The problem of the elitist research institutes (p.589) is by contrast not a problem, nor is the government's pursuit of space applications and nuclear energy. Not, that is, in India. The view from the West that activities such as these must be counted luxuries while poverty, squalor and needless death persist is not widely shared in India. The explanation is partly emotional: these activities help to lift spirits that would otherwise be profoundly depressed. But there is some logic, too. The scale of the social problems is so vast that even closing down the Atomic Energy Commission would not begin to scratch the surface. Indeed, when the social needs are for adult literacy, primary education and the other probable remedies for rapid population growth and the poverty that follows, the physicists working on the high-technology projects have little to contribute except the chance that what they do will bring economic success some day.

If the patronizing West insists on finding a contradiction, it is less stark than that between high-technology and rural poverty, and lies in the inconsistency of the policy of self-reliance. That is a recipe for spreading resources thinly and thus for doing everything a little less than excellently. The universities need most of all reform. The telephone system hardly functions. Can this be the long-term interest of the poor? □

Administration

How India runs its science

SCIENCE has a special place in the structure of the union government. While each of the fifteen ministries has operational responsibility for and need of science and technology (and some, like those for energy and irrigation, are largely technical in function), there is also a group of six agencies, without administrative responsibility except for themselves, which for practical purposes report directly to the Prime Minister. These agencies, whose influence on research and development is crucial, are listed here:

Atomic energy. Now responsible for the building of nuclear power stations and fuel manufacture as well as for research, this department also controls the **Atomic Energy Commission (AEC)**, with its headquarters in Bombay. During the chairmanship of Homi J. Bhabha, AEC established the nuclear research centre at Trombay (north of Bombay), now the **Bhabha Atomic Research Centre (BARC)** and was also responsible for creating the **Tata Institute of Fundamental Research (Bombay)** and a fuel production research centre at Hyderabad. Bhabha's successor, Vikram Sarabhai, inspired the collection of space-related institutes in Gujarat, first the **Physical Research Laboratory (p.600)** and the **Space Applications Centre**, from which the space enterprise has emerged.

Space. An outgrowth of AEC, the Department of Space now administers the **Indian Space Research Organization**, itself responsible for laboratories and launching facilities, the **National Remote Sensing Agency** and — separately — the **Physical Research Laboratory**.

Electronics. This department is also an outgrowth of AEC whose origin was the Electronics Council set up in 1970 under Professor M. G. K. Menon, who was then the director of the Tata Institute of Fundamental Research. As a department, the organization has responsibility for research work in electronics and communications and administers nationalized industries in electronics as well as safeguarding users in the field by way of standards.

Science and Technology. This department has grown in size and influence in the past ten years. On the advice of an independent council, it makes grants for research to universities and other institutions and also has a supervisory role in relation to the **Council of Scientific and Industrial Research** (which is formally separately responsible to the Cabinet).

Ocean Development. This now independent department has responsibility for oceanography both in research and diplomatically (through Indian interests in international treaties).

Environment. The newest department (created in 1980) has also been derived

from the Department of Science and Technology, partly because of the growth of the administrative burden of environmental legislation and partly because of recent interest in the politics of the environment.

Other organizations with a direct interest in science, technology and the universities include the four research councils, three of them dating from before independence (**Indian Council of Medical Research**, **Indian Council of Agricultural Research** and the **Council of Scientific and Industrial Research**). Like the **Indian Council of Social Science Research**, they represent an earlier phase of direct Cabinet responsibility for science and technology. The corresponding ministries have a supervisory role in relation to the research councils.

The universities are the responsibility of the **University Grants Commission (UGC)**, which has a supervisory function over universities and which makes grants for capital developments as well as financing central institutions directly (p.596). But the **Indian Institutes of Technology (p.594)** are separately financed through the Ministry of Education, to which UGC is also responsible.

To outsiders, the most distinctive feature of the Indian administration of science and technology (as of other matters) is the **Planning Commission**, a reminder that the constitution (adopted formally in 1950) embodies provisions for the systematic planning of the Indian economy on socialist lines. The next five-year plan will be in operation a year from now, to which end all research departments and the administrative entities to which they belong are now in the throes of drawing up proposals to be included in the plan. While the responsibilities of the scientist member of the Planning Commission, Professor M. G. K. Menon, extend to other fields than science and technology, his influence within the Indian scientific community and with the government suggests that the next five-year plan will be unusually interesting.

The scale of spending on science and technology in India has increased rapidly since independence and, in the current (sixth) plan amounts to 2 per cent of all public expenditure by the states as well as central government. Spending by the scientific agencies consumes 70 per cent of what is spent on science and technology, of which atomic energy and agriculture are the biggest spenders (23 per cent each in 1980-81). Medical research was 3 per cent of agency spending in the same year. UGC spending on research projects in universities exceeds Rs15 million, that of the Department of Science and Technology nearly Rs500 million. □

Antarctic research

Frontier in the far south

INDIA'S Antarctic exploration programme, which has mounted expeditions to the Princess Astrid Coast in each of the past three seasons (including that now ending) and has now established a permanent base, has one great asset — public enthusiasm and approbation second only to that for the space programme.

Why this should be so is not obvious. The cost of mounting the expeditions is high, with ships chartered from Norway and Finland, and the only obvious economic advantage is that the weather pattern of the Indian Ocean, and hence of the monsoon, may be determined in this region of the Antarctic. A report on the programme, published in January 1984 by the Indian National Science Academy, indeed suggests that this concern with climate influenced the Indian Government to go ahead, after people had been floating the idea for some years.

In reality, the government seems also to have been influenced by the putative wealth of Antarctica, and by the fact that the Antarctic Treaty expires in 1991. India, the largest non-aligned state, often shoulders a watching brief for other developing nations and was not prepared to allow the developed world to keep Antarctic resources for itself. But to carry weight in the Antarctic Treaty organization, a brief toehold on the Antarctic ice-shelf is not sufficient. There must also be a substantial scientific programme.

The first expedition in 1982, mounted in only four months, stayed only 10 days towards the end of that Antarctic summer. It made some meteorological and geodetic observations and carried out preliminary experiments on radio wave propagation, but most of the first harvest of data related to the sea-voyage. A major sea-mount, rising from a depth of 4,500 m to about 1,200 m was discovered at 53° 39.79'S, 47° 55.82'E and named, inevitably, Indira Mount.

The highlight of the spell onshore was the discovery of an "oasis", at 70° 45' 13"S, 10° 38' 14"E, where the unmanned Dakshin Gangotri weather station was established. Four fresh-water lakes were discovered in this area, with a high bacterial flora count (12×10^4 to 5.9×10^6 ml⁻¹) predominantly *Bacillus*, *Micrococcus* and *Corynebacterium*. A petrophilous lichen (*Acarospora* sp.) and a moss *Brymn* sp.) were found growing on the rocky substratum and in crevices in the area.

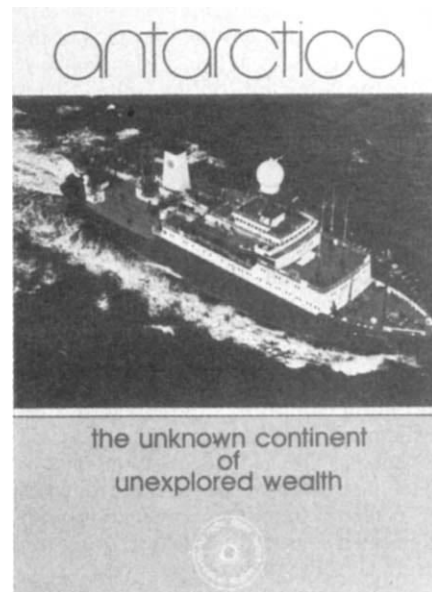
The second expedition, during the southern summer of 1982-83, spent 57 days on the Antarctic landmass and was able to make a detailed geological survey of 4.5 km² in the Dakshin Gangotri hills, and also a geological reconnaissance in the Wohlthat mountains. Snow and ice studies were made far inland into the Dakshin

Gangotri hills as well as recordings of snow accumulation and ablation of the shelf, changes in the surface microrelief and thermal profile of the shelf ice, and snow stratigraphy and density studies of the polar ice. Experiments were carried out on the artificial ablation of the shelf ice, and ice-core samples collected for dating purposes.

The meteorological programme of this expedition included the launching of 55 balloons (including 8 low-level sondes, 6 ozone sondes and 10 omega sondes). Radiation observations revealed strong reflection of solar radiation from the snow, without significant attenuation of ultraviolet in the reflected radiation.

The primary productivity of the Dakshin Gangotri lakes was estimated and measurements made of krill biomass at 16 locations. The magnetic programme included both a total intensity survey over a relatively small area using a proton precession magnetometer and the collecting of rock samples for palaeomagnetism. Radio propagation studies were continued, and the marine programme expanded to include acoustic and hydro-acoustic studies of the pack ice, and the drift of icebergs.

This impressive research package was



An Indian view of Antarctica — a booklet produced by the Indian National Science Academy, New Delhi.

sufficient to win India (along with Brazil) its desired seat on the Antarctic Treaty organization. But now, it is felt, there has to be a permanently staffed base from which observations can be made throughout the year. Of the 82 people comprising this year's expedition, a dozen or so will spend the winter there. **Vera Rich**

Oceanography

Playing for an ocean's wealth

INDIA'S National Institute of Oceanography (NIO) is appropriately situated in Goa, one of the most scenic areas of the Indian coastline and with a long maritime tradition dating back throughout its five centuries as a Portuguese colony. Yet in 1962, India's participation in the four-year international Indian Ocean research project was run from an office in Delhi. Goa, of course, had then only recently been incorporated into the republic. Only in 1969 were the NIO headquarters moved to Dona Paula, a promontory 7 km from the Goanese capital, Panaji.

Today, NIO also has regional centres at Cochin, Bombay and Waltair and two ships, the 1,900-tonne *RV Gangesani* and the 1,555-tonne *RV Sagar Kanya*. It has a staff of around 500 and comprises eight main research divisions: physical, chemical, biological, oceanography, geology and geophysics, instrumentation, ocean engineering and the planning and data division.

Like all CSIR institutes, its aims are closely linked with the needs of the Indian economy, ranging from development to marine farming techniques, and from pollution control to the extraction of drugs from marine organisms. NIO organized

the first Indian Antarctic Expedition, which was led by its former director, Dr S.Z. Qasim. (The Antarctic programme has since become a separate entity and in the opinion of some NIO scientists is distracting attention from the achievements of NIO itself).

But the institute has a number of recent successes to its credit. In 1981, for example, it found polymetallic nodules in the Indian Ocean and was then put in charge of the exploration of deep sea mineral deposits in the central Indian Ocean. The future share-out of these deposits is a politically sensitive issue for India, and NIO is accordingly proud of the speed with which it was able last year to negotiate two possible sites for exploration with the UN authorities, as required by the Law of the Sea treaty.

Equally important for India's policy of self-reliance is the work on extracting organic chemicals, including pharmaceuticals, from marine life. This has been carried out on a somewhat pragmatic basis; marine flora and fauna have been collected, and tested for biological activity. There have already been some notable successes with biota from the continental shelf. Anti-hypertensives and even contraceptives may result. A new joint Indian-

United States programme which will extend the search beyond the limits of the continental shelf was launched in January. (The funds for this, on the United States side, come from the Indian repayment, in rupees, of past United States loans for food aid.)

Less exotic, but equally important for the Indian economy, is the aquaculture programme, including shrimps and mussels, as well as local fish such as *Etropus suratensis* which, it has been found, can be successfully farmed in the canals of coconut groves. Recent projects include the induced spawning of mullet and the mass culture of feed organisms — nematodes, turbellarians and harpacticoid copepods. The biology division investigates the oxidizing and reducing properties of bacteria found in conjunction with manganese nodules, which play an essential role in nodule formation.

NIO's main cause for rejoicing during the past year, however, was an event which happily did not take place. The west coast



A glimpse of the goal. . .

of India lies full in the path of currents from the Arabian Sea, and is therefore always at risk from tanker spills and discharges. Last spring this hazard was immeasurably compounded when a vast slick, discharging from oil-wells bombed in the Iraqi-Iranian war, began moving down the Persian Gulf and out into the sea. Specialists in ocean currents and chemical oceanography laid their contingency plans for a major devastation — and waited. In the event, the slick dispersed in mid-ocean, and NIO, in retrospect, can refer to the alarm as “instructive”.

Vera Rich

Japan and the United States. Perhaps in 1990 we shall be only five years behind.”

This outsider's impression (see elsewhere on these pages) is that both Sidhu and the critics of his organization have a sustainable case. The National Chemical Laboratory at Pune is, indeed, widely respected for the design of heterogeneous chemical catalysts, the National Physical Laboratory (in Delhi) does masterminding the imaginative Middle Atmosphere Research Programme (an outgrowth of the laboratory's long-standing interest in radio propagation and the ionosphere) and the Centre for Cell and Molecular Biology at Hyderabad is a brave adventure in basic research.

On the other hand, there are projects and programmes in CSIR laboratories which are imitative of work being done outside India but which are unlikely to make a distinctive mark in the foreseeable future. And some research projects are simply second rate. Much obviously depends on the directors of individual laboratories. Much depends on the quality of the staff they inherit, and on those people's willingness to change their ways. But there are vivid demonstrations, at the National Geophysical Research Laboratory and the Centre for Cell and Molecular Biology (both at Hyderabad) that a determined director can pull a programme round to his way of thinking in spite of the bureaucracy.

Would it not help if there were stronger links between CSIR laboratories and the outside world? Sidhu is planning to move in that direction. CSIR will listen to the Public Accounts Committee's complaint that research advisory committees appointed for each laboratory will in future meet regularly, and he hopes to strengthen links with universities by setting up some research units on campuses of which the Institute of Microbial Genetics set up at the University of the Punjab may be a prototype. The council has also approved a scheme to allow university people to work at council laboratories as well as to encourage laboratory staff to teach.

The bureaucracy is not, Sidhu insists, nearly as great a problem as the critics say. Because India is a parliamentary democracy, the director-general and his staff must know what is going on in the laboratories. But for the past two years he has been trying to persuade laboratory directors to take seriously the manual on delegated authority he put out in 1982. His hope is that by the time of the seventh five-year plan (to begin in 1985) it will be possible for CSIR substantially to have “watered down” its role as the final arbiter of the details of all laboratory research programmes.

For the future, Sidhu hopes that CSIR will continue to emphasize the importance of basic research. Originally, he notes, the chief objective was “import substitution”. Now, he thinks, the time has come when CSIR must hope to “come up to some of the world's best”. Concentrating resources

Industrial research

Source or victim of red tape?

THIS has been a bad year for Dr G. S. Sidhu, the director-general of the Council for Scientific and Industrial Research (CSIR), India's chief agency for turning science into industrial prosperity. At the end of last year, the Public Accounts Committee of the Indian Parliament prefaced a critical report on the working of the organization with the flat declaration that it is “disappointing that CSIR had failed to make any significant impact on the development of technology for use in industry” and, in the process, implicitly raised questions about the future of the organization.

CSIR's origins go back before independence to 1942, and to the belief that the economic future of India would turn on the successful application of research in manufacturing industry. Now, the council's spending amounts to more than Rs1,000 million a year. CSIR maintains 35 national laboratories whose programmes collectively range from basic research to strictly practical projects. It is also one of the chief means by which young scientists can hope to find employment in India, partly because of its fellowship schemes by means of which people can be kept in research for limited periods.

The Public Accounts Committee's complaints were several. The proportion of CSIR projects taken up by industry is too small (15 per cent or less), too little attention is paid to low technology that might benefit the rural poor, CSIR runs pilot plants (for the production of optical glass and magnesium for example) uneconomically and outside its terms of reference, younger scientists have too little influence on the programmes of the

laboratories in which they work and — scandal, scandal — two separate laboratories were found to have embarked on the development of a household water pump in the 1960s without the central administration being aware of what was happening. (CSIR's defence is that the cost of the duplication was only Rs10,000, and that it has nearly 2,500 projects to monitor.)

The report is personally hurtful to Dr Sidhu, coming as it does on the eve of his retirement (in May) from a post he has occupied for nearly a decade. And in any case, he says, the record is not nearly as bad as it seems. In 1983, he guesses, more than Rs600 million was invested by the chemical industry alone in processes developed at CSIR laboratories, yielding products as different as anti-inflammatory drugs and for manufacturing methyl acrylate. The laboratories are a principal source of microwave systems for defence, while the council's capacity to move quickly is illustrated by the way in which it surveyed 300,000 square kilometres of the Indian Ocean in only four months last year, when asked to advise on sites at which manganese nodules might be mined (see above).

One of the council's mistakes, Sidhu says, may have been that it has done less to publicize its successes than it might have done; “we are trying to modify that”. But CSIR must also remain an agent for basic research. Silicon technology is an example — CSIR laboratories have the knowhow on which a microelectronics industry could be built, but whether such a technology will emerge depends on the government's willingness to invest in it. “Now”, says Sidhu, “India is 10 or 15 years behind industry in

on good projects is one way of doing that, which is one reason why CSIR projects as a whole have been reduced by a third in the past few years.

Import substitution, however necessary it may in the past have been, has probably become a distracting objective for an organization such as CSIR, now conscious of the importance of quality in research, if only because it justifies imitative and repetitive research.

Another difficulty is that CSIR, firmly placed within the government structure as it is, cannot be sure that the research projects it carries out for the benefit of Indian industry will yield products that industries

Pushing invention

FOR the exploitation of inventions arising within the public sector, India has a National Research Development Corporation modelled on the same-name British organization now absorbed in the British Technology Group. Out of an income of some Rs13 million a year, the corporation returns 60 per cent to the inventors. Some development is carried out directly: the corporation is building solar heating plants on the roofs of two Delhi hotels and taking an equity stake in an electrolytic manganese dioxide plant. Dr H.S. Rao, the managing director, says that the flow of inventions offered is nevertheless declining.

will actually wish to exploit, for which reason the Public Accounts Committee's complaint that only 15 per cent of CSIR projects were actually exploited may be less discreditable than it seems. But although everybody seems to agree that manufacturing industry has recently become much more conscious of the value of research, as shown by the growth of contract income at CSIR and other government laboratories, experience elsewhere shows that networks of industrial advisory committees are much less effective in ensuring the relevance of research than a company's tangible commitment to a project represented by its own investment in it.

The conditions on which scientists are employed in CSIR and other government laboratories must be, as it seems to an outsider, another handicap. The principle seems to be that an appointment to CSIR is an appointment for life. There are rules for promotion from one grade to another which usually require that a person should have spent five years or so in his post, disappointed employees can appeal if they are not promoted when they might be, everybody is badly paid (laboratory directors may have a gross salary, including allowances, of Rs4,000 a month, people recruited with a first degree less than a third as much) and there is no sense among new recruits that they will become powerful figures in their laboratories, perhaps even directors, if the quality of their work is high. The government service is too grey for its own good. □

Geophysics

Resources of a moving plate

THE National Geophysical Research Institute may yet prove to be an illustration of how, even in India, it is possible to take an established institution by the scruff of the neck and point it in a different direction. The laboratory, in a handsome set of modern buildings at Hyderabad, is part of the CSIR network whose objectives stem from the argument that India must be stuffed with natural resources and that a geophysical understanding of the subcontinent is a necessary basis for discovery.

The new director (since last April), Dr Vinod Gaur, is an energetic youngish man from the University of Roorkee, originally a technical college which has in the past ten years won a high reputation as an engineering school. Gaur does not know whether the life of an administrator will suit him — he knows he enjoys tramping about in

the northern foothills looking for places at which to site seismographs. So he has agreed to run the laboratory at Hyderabad on a five-year secondment from Roorkee.

Gaur's arrival at Hyderabad will be long remembered. He began by telling the 180 scientist members of the staff that they should spend the following three months in the library, reading the recent literature in their fields, deciding what they should be doing. In Gaur's opinion, the underlying difficulty in the management of government laboratories is that people have no incentive to be active. It is easier, he observes, to "recycle" the same idea over and over again.

On the basis of a series of workshops held at the laboratory last summer, he says that it has now been possible to hammer out a coherent programme of research. The laboratory will take up anew geochemistry (there is a high-performance mass spectrometer on order), deep seismic surveys of south India and earthquake prediction. Gaur says that CSIR has backed him up in his deliberately subversive spell as director, and that he hopes to be able to appoint 30 new people to the staff in the course of this year, partly because of planned retirements and partly on the strength of new money.

The tangible outcome of this approach so far has been a thick two-volume plan of what the laboratory wishes to undertake in the years ahead (and a corresponding bill for CSIR). The programme is ambitious, nothing less than the use of modern geophysical methods for a full description of the Indian lithosphere and its surroundings.

The hunt for minerals, oil and natural gas especially, is at the back of everybody's mind. In this connection and others, the geophysical laboratory is the basic research arm of a group of interested Indian agencies. Some support for this work comes from overseas organizations, the UN Development Program for example.

Instrumentation is an important part of the programme, and the laboratory plans both to improve on existing instruments (and stimulate their manufacture in India) and to develop a computer centre capable of handling efficiently the huge volume of data generated by geophysical prospecting.

Much of the general interest in the laboratory's programme stems from the continuing northward movement of the subcontinent relative to the Eurasian plate, in which connection the results gathered by the Franco-Chinese expedition to Tibet (see *Nature* 307, 17-36; 1984) are of special interest. But deep seismic surveys organized from Hyderabad have now thrown light on another Indian geophysical puzzle — the origin of the Deccan Traps, the huge lava flow that bites into the west coast of India over a distance of 200 kilometres and

Defence shows off

DEFENCE science, normally as reticent in India as elsewhere, marked its silver jubilee this year with a small exhibition in Delhi on Army Day (14 January). And a series of annual awards to "defence scientists of the year" has been instituted in fourteen fields.

This year's "silicon trophy" is shared by five laboratories which contributed to the development of India's first indigenous main battle tank (now in its final development stage). Second place (the "titanium trophy") has gone to the Defence Food Research Laboratory, Mysore, for its development of processed foods for Antarctica and for the Indian visit to Salyut-7. Other awards range from the conventionally military (ammunition and camouflage for example) to such unlikely topics as high-altitude agriculture, poultry and animal husbandry — a reminder of India's constant concern with the Himalayan frontier.

A similar mix was to be seen in the exhibition. Alongside sophisticated electronics concerned with the performance of projectiles and explosives were protective clothing for mountain troops, mosquito, fly and leech repellants for the south, a dry body-shampoo for desert conditions and survival ration packs (vegetarian).

The relationship between defence and civil science is far from obvious, but it may be significant that laboratories operated by the Department of Defence Research and Development Organization are often sited alongside their opposite numbers in the civil sector. Thus the Defence Food Research Laboratory and the Central Research Food Technology Institute are both in Mysore and the Explosives Research and Defence Laboratory and the National Chemical Laboratory are both in Pune.

Vera Rich

which reaches as far inland.

Dr K. L. Kaila, leader of the seismic sounding programme, explains that data accumulated in the past few years have shown that this igneous structure, perhaps the largest accumulation of lava now known, consists of a lens-shaped structure approximately 1.5 km thick on the west coast which becomes progressively thinner as it is followed inland. The inference is that the source of the material lies seaward of the west coast, perhaps at the edge of the still moving Indian plate.

The practical importance of this investigation, first stimulated by the Oil and Natural Gas Commission (one of the sponsors for the continuing investigation) is the discovery beneath the igneous layer of a sedimentary basin perhaps 100,000 km in

extent which is itself up to 2 km thick and which is almost certain to have been formed when India was still part of Gondwanaland. There is now high hope that there may be substantial economic reserves of coal beneath the relatively thin igneous overburden where the Deccan Traps thin to a few hundred metres towards the east. Oil and gas would be even more welcome.

Among CSIR laboratories, the geophysical laboratory is perhaps the most stringent test there could be of the combination of basic research with humdrum management. Shooting a seismic profile requires not merely instruments and means of gathering the data they produce but a small army of truck drivers and other miscellaneous helpers. Mercifully for Gaur, there is no shortage of them. □

Molecular biology

Ambition unrestrained

In the heady days after independence from British rule, one of the first of the CSIR laboratories to be created was the Regional Research Laboratory (RRL) at Hyderabad, now a particularly Indian proof of the principle that it is much easier to found an institution than to close it. But, like a chrysalis from which a butterfly emerges, the laboratory has given rise to one of the most talked of laboratories in India. Bhargava, people say, "can get away with murder".

The reference is to Dr Pushpa Mitra Bhargava, the director of the Centre for Cellular and Molecular Biology which has been quarried by the dint of unspoken politicking from the dormant body of RRL. Dr Dorairajan Balasubramanian, the deputy director of the centre says (Bhargava was in Lucknow for the week): "Our aims are up here" (lifting his hand above his head). "We want this laboratory to be as well known as the Salk Institute or the Medical Research Council Laboratory at Cambridge."

The centre, formed in 1979 but now housed separately from RRL on a separate campus a mile away which is dominated by new construction, is aiming deliberately at that goal. The scientific staff now amounts to sixty, organized into half a dozen groups. Ultimately there may be three times as many. But in spite of the extra space there will soon be, the senior people insist that recruitment will be deliberate.

Younger people, for example, are recruited by means of an advertisement in the national press every six months. Each round produces 300 applications from new PhD graduates, of whom perhaps 100 are called for an interview and for an ingenious elementary test of scientific competence — what, for example, is the function of the ball in a ball-point pen and is a sea horse a mammal, reptile, fish or invertebrate? Applicants are invited to answer 60 questions in two hours, and it seems that on many occa-

sions all those called to Hyderabad go away without a fellowship.

The same principle applied to more senior appointments — the laboratory has set out first to recruit people with such a well-established reputation in some field of cell or molecular biology that they can become project leaders, and then to give the people concerned an important voice in the choice of those who work for them.

By Indian standards, such a policy is bound to seem undemocratically discriminatory. Why should one man's PhD be preferable to another's? The answer is that a productive laboratory requires well-articulated teams of people. And in the present mood in India, when it seems to be dawning on people that excellence is not merely desirable in itself but the only sure foundation for worthwhile discovery in a competitive field, breaking convention may be necessary.

Certainly the centre seems to have won the support of the people at CSIR headquarters. Over five years, the laboratory will have spent Rs350 million, mostly on buildings and equipment. The NMR machine on order will cost Rs5 million, the cell sorter nearly a tenth as much.

Technically, the laboratory has much to boast of already. Balasubramanian, a physical chemist by origin, is for example one of the few practitioners of the craft of photo-acoustic spectroscopy — the technique by means of which a laser is used to excite characteristic molecular vibrations. One neat application for example makes it possible to detect in malaria parasites complexes between breakdown products of haemoglobin and the drug chloroquine, themselves believed to be toxic to the parasites (see *Science* 223, 828; 1984).

The research programme as a whole appears to consist of projects of two kinds — those to which an Indian laboratory can make a distinctive contribution (as in the molecular basis of the sense of smell, a

mouse-based genetic study, and the search for novel ribonucleases such as that known to occur in human milk, a project with an Indian interest because of the lower concentration of RNAase in the milk of Parsee women who are also prone to mammary cancer) and mainstream molecular biology, genetic regulation and the role (if any) of retroviruses in oncogenesis.

If they have their way, the project leaders will stick to basic research, resisting the temptation to become the mainspring of biotechnology for the subcontinent. But the laboratory would like also to be a source of trained people for the biotechnology enterprises which the Government of India is doing everything it can to foster.

In the long run, it seems agreed, mobility is what will matter most. If "Bhargava's place", as the laboratory is also widely known, can win a reputation for itself and at the same time not be so attractive that the people whom it recruits with such care are never prepared to leave, its founders' high ambitions may be fulfilled. Meanwhile, as

Killing mosquitoes

THE ponds of the campus of the University of Madurai are uncommonly free from mosquito larvae, thanks to repeated spraying with a bacterial larvicide secreted by sporulating *Bacillus sphaericus*. The aim is to use the biocide as a complement to chemical insecticides in breeding grounds for *Culex* and *Anopheles* mosquitoes.

Dr Kunthala Jayaraman, sometime Harvard post-doc, explains that the toxin lasts well in spores released in the field, that genetic selection of bacteria should yield strains with amplified genes and that (in collaboration with the CNRS laboratory near Paris) the gene has in any case now been cloned in *Escherichia coli*. The toxin is effective at the microgram per ml level. □

in any Western laboratory, the senior people reckon to help determine policy corporately, and insist that time spent on group seminars is part of the job.

The new centre's parent, RRL, was no doubt once like that, and even now it suffers by Indian standards only by comparison. Essentially an applied research laboratory, RRL's programme has not changed much in thirty years. The processing of minerals is a large part of the programme, the exploitation of natural vegetable oils another.

The laboratory commissioned a Lurgi coal gasification plant last September, aimed largely at the exploitation of the lignite coals of the region. The chemistry of vegetable oils continues to spawn formulae for new drugs. The economics of the vegetable oil industry, a constantly shifting quagmire, preoccupy the managers. With its 1,200 people, however, the laboratory may well be too large to manage effectively, or to be effective. □

Environment

Search for a policy

DEVisING a policy on the environment that will be soundly based in science is the task that Dr Triloki Nath Khoshoo, Secretary to the Government of India at the Department of the Environment, has set himself. Khoshoo is a plant geneticist persuaded reluctantly to Delhi only after eighteen months of pressure from "the highest levels". He had been a distinguished director of CSIR's National Botanical Research Institute at Lucknow, once the National Botanic Gardens but now much more concerned than that name would suggest with the propagation and cultivation of vegetable and medicinal plants as well as flowers.

Khoshoo has arrived energetically in Delhi proclaiming that he is not a "miracle man". While his department (created after the 1980 election in which environmental issues had figured prominently) has executive responsibility for the air and water pollution legislation and wildlife protection legislation of the 1970s, Khoshoo seems to be especially interested in the power his position offers to keep an eye on developments planned by other departments and, if necessary, to fight them in cabinet committees. He is keen that all major projects should be looked at. He seems undismayed that his role is a recipe for making enemies.

If Khoshoo has his way, India may be the first country of any size to have an environmental policy securely based on Darwinian principles. Too many discussions of environmental and conservation questions, he says, are based "more on fiction than on fact". Khoshoo keeps reminding his listeners that any ecosystem is in some kind of dynamic balance or imbalance, and that conservation cannot be a literal process.

The astonishing diversity of India is inevitably a complication for conservationists. From the equatorial Andaman Islands in the Bay of Bengal to the Himalayas is a huge ecological step. Largely with the spur of the Unesco programme "Man and the Biosphere", however, a dozen representative sites have been identified as potential "biosphere reserves". Part of Khoshoo's job in the years immediately ahead will be to win the approval of state governments for these plans, somehow to reconcile regional vested interests and then to win the funds from the central government that will be required for operating the reserves.

He has more immediate conservation problems on his plate, however. Setting up five gene banks for plant material would seem a straightforward task, but one gathers that it has not always been a simple task to persuade technicians to take responsibility for commissioning imported deep freezes. And there is the contentious issue of the forests.

The Indian love affair with trees is

matched only by the waywardness with which farmers and one-cow families allow their animals to graze whatever land has traditionally been open grazing ground, with disastrous consequences for saplings and young tree growth, and by the zeal with which the rural population scavenges for fuelwood. More recently, fierce passions have been roused by the introduction into India of *Eucalyptus* species, which grow splendidly in much of India, which are described by their supporters as a necessary source of fuelwood but which offend conservationists (because of the competition they offer native species), nationalists (because they are exotic) and ecologists (because they lower the water table).

Khoshoo is doing his best to help cool this argument. In January, he told a conference on eucalypts in Kerala that it was clear government policy that exotic trees should not be introduced into conservation areas, but that farming eucalypts on tracts of land that would otherwise be devoid of forest can be not merely a direct economic benefit but could also help to relieve pressure on natural forests. But "there is a need for more scientific data" about the circumstances in which *Eucalyptus* can be grown without environmental damage, which is another way of saying that those who wish may delay, and the whole dispute should in any case be set against a policy of afforestation — in 1952, the government decreed that a third of India should be covered with trees, but the proportion has instead fallen to 10–12 per cent — and of plant breeding to improve forest production. Meanwhile, even in the streets of Delhi, people carrying bundles of twigs and branches culled from city trees can be seen making their way home. The foresters have a battle ahead of them. Inevitably, Khoshoo is a plant-orientated environmentalist ("How can you have animals

without plants?") and hopes to use some of his department's research budget for supporting studies in seed biology, the use of tissue culture techniques and the like. But he will manfully prosecute "Project Tiger" and help encourage the breeding of crocodiles. Given that nearly half the land surface of India (part desert, part mountain) is unused, Khoshoo hopes there will be a number of environmental institutes dealing with specific regions (the Himalayas are first on this list).

Among pollution problems, water pollution (in its broadest sense, the most common cause of death in India) is paramount. But in the cities, the chief cause of pollution is dust (as anybody who runs his finger along a piece of Indian paintwork can tell). Concentrations of dust range from 50 µg to 1,000 µg per m³, making this land of open skies one of the grubbier in the world. So Khoshoo will be taking on big industry and small business, but not yet. Hard-headed cost-benefit analysis is for the time being the order of the day, all within a framework of some 500 separate pieces of legislation and with the hope that state governments can be persuaded to do what is required of them.

Perhaps, unknowingly, Khoshoo is as it happens a natural politician with a thoroughly un-Indian regard for the self-respect of his junior colleagues. Like natural politicians anywhere, he is building a constituency by means of an imaginative programme of public awareness, chiefly among schoolchildren and their teachers. Last year, he was able to give 140 prizes for school projects of an environmental character — making a model of an elephant for example. This year, no doubt, there will be more.

Making what is generally known as ecology scientifically respectable is Khoshoo's other goal. Perhaps there will be an Academy of the Environment, but certainly there will be a research programme, perhaps even a big one. And Khoshoo has every chance of remaining what he is now, a bureaucrat talking like a scientist. □

Prizewinners

No more Indian heroes

WHERE have all our Nobel prizewinners gone? This constantly repeated question is not satisfactorily answered by mentioning Professor Subrahmanyan Chandrasekhar, the astrophysicist who shared the physics prize last year, for he works in the United States and is in any case too modest a man to wear with ease the mantle of greatness that India is bursting to thrust on its heroes. Now Bose and Raman were different . . .

C.V. Raman's reputation is kept alive in Bangalore, in the institute which he founded there in the 1940s in a piece of intellectual entrepreneurship comparable with, say, Sir Humphry Davy's foundation of the Royal Institution in London more than

a century earlier on the generosity of his admirers. From his retirement as director of the Indian Institute of Science until his death in 1970, Raman's institute was his personal research fiefdom where, with a succession of assistant devotees, he constantly sought the unattainable — a second discovery comparable in importance with the Raman effect of the 1920s. An upper room at the institute filled with the objects collected for scientific observation — spectacular mineral crystals predominate — is his shrine.

The Raman Research Institute is now a very different place, a physical research laboratory with two concentrated research

programmes supported almost entirely by the Department of Science and Technology. (Raman's endowment has withered away to 1 per cent of the total income.) The director of the laboratory, Dr V. Radakrishnan, is a radioastronomer (with an Australian accent) whose chief objective seems to be to bring the temper of Western science to southern India. The radiotelescope at Ootacamund, nearby, is used for observations of pulsating stars; that at Gauribidanur (operated in conjunction with the Indian Institute of Astrophysics) operates at decametre wavelengths and is one of the largest of its kind anywhere in the world. There is a plan to design and build a millimetre wave telescope.

Alongside this programme in physical astrophysics is an outwardly incongruous group — Professor Sivaramakrishna Chandrasekhar's liquid crystal team. By general agreement (outside India), Chandrasekhar's own contributions to this field have been outstanding. He is a refugee in Bangalore from the University of Mysore, where "there was no library and no laboratory space". His group of 20 people is as much concerned with applications as with theoretical understanding — but Chandrasekhar himself is disappointed that after he had blazed "an inconspicuous trail" in the 1960s towards the use of liquid crystals as visual displays, the agencies of the Government of India were reluctant to believe his claims until RCA in the United States had shown them to be realistic. Now the institute has made an arrangement with a private company for the use of his latest liquid crystal display in the manufacture of a million watches, one of which he wears proudly on his wrist.

This institute is as vivid a proof as there could be that research of the highest class is alive and well in India. The necessary conditions seem to be that people of distinction should work in institutes which are small and generously endowed and that it should be possible for those with ideas freely to select those who work with them. The benefits? Reputation, some practical benefits (Chandrasekhar's work for the Defence Department as well as his watch display) and a trickle of people with distinctive PhDs.

Calcutta, the other pole of physical science in British India, has its own institute on this pattern, but to commemorate J.C. Bose, not S.N. Bose (of Bose-Einstein statistics) but the Cambridge educated contemporary of Lord Kelvin who, in Calcutta, divided his attention between classical physics and plant physiology. (S.N. Bose, educated in Calcutta, moved to Dacca in 1921.)

His institute, a cloistered gothic building (with ivy) is in the throes of moving to a new site on the way to Calcutta Airport. Like the Raman Research Institute, it has an independent programme of research in which nuclear physics is prominent, but which runs to biology as well. Again the

objective is to train exceptional graduate students but, Calcutta being the culturally self-conscious place it is, the Bose Institute thinks it proper that it should tell the world (or at least West Bengal) what science is about.

The Indian Association for the Cultivation of Science in Calcutta has similar but more explicit terms of reference. Founded in 1876, and the place at which Raman began his work, the association is partly a membership organization and partly a research institute. Funds come from the Department of Science and Technology and (in smaller amounts) from the Bengali Government. The research runs from theoretical physics to the experimental study of macromolecules. With a staff of 80 qualified people, the association produces more than a score of PhDs a year.

Like the Bose Institute, it also serves as a central repository of research equipment, running a helium-liquefaction plant for the benefits of neighbouring universities and institutes. The director, Professor A. K. Barua, a solid-state physicist by background, has turned enthusiastically to a project for improving the photoelectric yield of amorphous silicon as his and the association's contribution to a nationally coordinated programme of research. (They hope to be able to attain 6 per cent efficiency.)

The lesson of these fundamental research institutes is not the obvious — that small is beautiful. The Tata Institute of Fundamental Research on the other side of the subcontinent is much larger but has the same temper (and is the best equipped). TIFR as it is commonly known is essentially a creation of the late Dr Homi J. Bhabha (its first director) in the 1950s, when it was very much an offshoot of Bhabha's Atomic Energy Commission. From the start, the objective has been to create a centre of excellence, and one whose international connections would be strong.

The result is an institution that most closely resembles those in the West typified by the Institute for Advanced Study at Princeton University, even to the extent that the director for the time being reckons still to be a working scientist. (Professor B. V. Sreekantan, the present director, is a cosmic ray physicist.) Members of the faculty are mostly professors of the institute. Most other scientists are either fellows on three or five-year fellowships or students following the PhD course (but registered at, say, the University of Bombay).

The research programme is ostentatiously catholic. Mathematics is strong, especially in algebra and group theory (with a continuing interest in the prodigious stream of conjecture left behind by Srinivasa Ramanujan, the young Indian mathematician taken up by G. H. Hardy of the University of Cambridge in the 1920s). Theoretical physics runs to the esoteric —

grand unified theories, for example — and theoretical astrophysics (which has J. V. Narlikar, Hoyle's ex-colleague) to "quantum cosmology". An experimental high-energy physics group which has operated a bubble chamber at CERN at Geneva has now joined a collaboration that will include a Chinese group to plan an experiment for the large electron-positron accelerator (LEP) due to be commissioned at Geneva in 1988.

The underlying strategy is that a laboratory like this must keep up with all the Joneses, at least in the quality of what is done. The nuclear physicists at TIFR, lacking a large accelerator of their own, use the variable-energy cyclotron at Calcutta but are building an ingenious accelerator of their own from a bought-in tandem Van de Graaff machine and a superconducting linear accelerator whose twelve accelerating modules have been fashioned on the premises. Delay in finding out about the properties of nuclei is counterbalanced by what is learned about an advanced technology.

So it is with the radioastronomy and the balloon and satellite-borne astrophysics — the scale of operations is less than it might be elsewhere, but the operations are qualitatively no different. But the pursuit of excellence is impeded not by the difficulty of providing equipment but by the personal difficulties of working in local isolation (TIFR's generous overseas travel budget notwithstanding), which is why the work of people such as Professor O. Siddiqi on the behavioural genetics of *Drosophila* may be as remarkable as anything else at the institute.

Inevitably, TIFR and other free-standing institutes like it are widely criticized (what, in India, is not?) as ivory towers, detached from the country's true problems. (Most of them have projects aimed at problems in development but the design of a rural windmill or cooking stove is hardly the price of a licence to be an academic of the international community.) So formal papers are written endlessly about fundamental research in developing countries. The most convincing argument is that the practice of international science in a country such as India is a proof to the others that it can be done, and thus an example to be followed, and that in due course these ivory towers will be invaluable when India's gamble that it has a prosperous future pays off.

How safe are these frankly elitist establishments? In the present climate in Delhi, there seems hardly a cloud on the horizon. As private foundations, they also enjoy the protection of the law (which does not, of itself, ensure their budgets). At present the most serious risk is that, in spite of the arrangements made for self-renewal, they may be undermined by the immobility that abounds. In the past fifteen years, even TIFR seems to have aged a little, not by the whole of fifteen years but perhaps by a third of that. □

Universities

The most chaotic education anywhere

THE Indian university system is certainly the largest in the world, but probably also the most chaotic. Some institutions are good. Most are afflicted by a host of problems — inadequate facilities, inadequate teachers, inadequate students, too many students, too much external political influence, academic nepotism (or even corruption), police brutality towards students, excessive concern for academic qualification and status, meaningless or even phoney research. The wonder is merely that so many young people survive the system, even wresting an education from it.

In round numbers, there are three million students at roughly 120 Indian universities — unambiguous definition is not easy — but undergraduate teaching at the

larger institutions is delegated to what are called affiliated colleges, of which there are nearly 4,500. Since independence in 1947, the system has grown with astonishing speed, by a factor of twenty or thereabouts when measured by student enrolments. Even so, the participation rate, the proportion of young people of the appropriate age finding their way into higher education, only a few per cent or so, is tiny compared with that in most industrialized countries.

For some of the faults of the system, Indians can fairly blame the British occupation of India. Indeed, the general pattern of the Indian university was fashioned by the British administration in 1857, which in that year created three universities (Calcutta, Madras and Bombay) by adapting the formal organization of what

was then the University of London to Indian circumstances. On this model, the university was simply an organization for setting examinations and for subsequently awarding degrees.

Administratively, this system had the convenience that it could handle steadily increasing numbers of students without the central administration being disturbed by the need to write new charters for new institutions. This same feature of the system has naturally made growth virtually uncontrollable in the decades since independence.

The same process is encouraged by the way in which financial responsibility for the universities rests with state governments (but seven institutions are supported centrally, through the University Grants Commission (UGC), while a few others depend directly on central government ministries). Local and regional demand for higher education cannot lightly be disregarded, while state governments may also calculate that the direct cost of operating a new university (or of expanding an existing institution) may be kept within reasonable bounds if others, charitable organizations or industrial companies, are persuaded to meet the cost of operating affiliated colleges. The consequence is that the UGC, which decides which universities can award degrees, has often been but a pawn in a game played between central and regional governments.

As things have developed, the larger federal universities are more than mere degree-stamping organizations. The doctrine that research should be a part of higher education, widely established in India after the First World War, has led to the arrangement that federal universities should maintain academic as well as administrative staff, should award masters' degrees and PhDs (after two and a further three years respectively) and that there should be a grant-awarding mechanism (through UGC and, more recently, through the Department of Science and Technology and other ministries) for the support of research.

The consequences, for the temper of academic life, are curious. Many departments in the better-organized universities have all the appearance of being research establishments, with graduate students working hard on the problems that engage the interest of their supervisors and with little to remind those concerned of the harsh realities of academic life — the need, in particular, that undergraduates should be well taught. That, one senses, is an administrative problem.

Thus one physicist at the University of Delhi (one of the seven centrally supported universities) complains eloquently that

College association makes a mark

THE difference between a pressure group and a ginger group is one of temperament. The earnest gaiety of the Indian Association of College-Going Scientists puts it squarely in the second category. It is a group of 2,000 bright science students whose objective is to persuade their elders to improve the quality of teaching in the universities, and meanwhile to persuade those same elders to give lectures on cosmology and molecular biology in Delhi and Bombay and who wage a running battle against superstition in general and astrology in particular. There is no record of them having *gheraoed* a vice-chancellor in his office.

The organizers are fresh-faced and neatly-dressed for revolutionaries. Indeed, their own recruitment literature calls them "budding scientists". Somehow they have persuaded bodies such as UGC and the Department of Science and Technology to help them out with modest grants, "a thousand rupees here and there".

The ring-leaders are able young men who bubble with enthusiasm for science and for their cause, the reform of the educational system. Once a year they organize an All-India Congress at which about half the speakers are invariably from the older generation who, in the setting in which they find themselves, almost always commit themselves to the reform movement.

N. Srinivasan, the secretary at the association, a Bombay MSc student in astrophysics, is eloquent about the need for change. His chief complaint is at the rigidity of the examination system; there is no credit for knowing something about science but only for what is in the syllabus, with the result that those who can regurgitate what they have learned at examination time perform well, while the

others are discouraged.

The paradoxical result, the association says, is that there is a need to popularize science among the audience that should need this least, science students at the universities. Whence the projects the association persuades its sponsors to let it undertake — after building a 30 cm nitrogen laser with the help of the Bhabha Atomic Research Centre, the Bombay chapter of the association now hopes to build a radio-telescope, with the help of TIFR (see p.590) at the Ootacamund centre.

The running battle with the astrologers (in which the association is supported by people like P. M. Bhargava, (see p.588) is not merely amusing. While astrology is well entrenched in India, few will speak out against it, leaving a fruitful field to the association. With a publicist's timing, the association put out a statement attacking astrology (and appealing to the press not to publish horoscopes) on 10 March 1982 — a day predicted by some astrologers to be a kind of doomsday. It earned from the *Astrological Magazine* the put-down that its president should address the "more onerous task of minding his own studies first rather than straying into unfamiliar grounds with a bee in his bonnet".

The association is both admirable and astonishing. Why are such well-educated people so angry about the educational system? Their critics say that they are merely practising the skills they will need when they, too, join the establishment — how to run membership societies, congresses and so on. But the distinction of their well-placed backers suggests the association has a more subtle function, that of making public criticisms of the educational system that most people utter only in private. □

One oasis of success in Bombay

ONCE-Portuguese Goa is not much more than 200 miles south of Bombay, which is part of the reason why a Jesuit educational foundation is one of the hundred or so constituent colleges of the University of Bombay. St Xavier's College was begun in 1869, soon after the university itself, but is hardly the place at which to see the weak link of Indian higher education, the down-at-heel affiliated college, at first hand. In reality, the college boasts that more than 90 per cent of those entering for their final undergraduate examinations are awarded degrees. In science subjects, more than half of the degrees awarded are in the first class.

The college is, however, a splendid illustration that high-quality education is possible even in gothic buildings which are chiefly a monument to the optimism of their Victorian architects. Worn stone steps have been fitted into the ornamental turrets, science laboratories have oriel windows, the library looks like a chapel and as much as possible of the downtown campus is covered with ivy. The Jesuits have the advantage of continuity.

Inevitably, the competition for entry is fierce. Selection is by the marks awarded in school-leaving examinations, but the college gives preference to those from Roman Catholic schools (and to the children of former students). Sport is applauded, and there is a two-year junior college for those needing to complete their preparation for a degree course.

Success seems to derive from the intimacy of the place. Dr (Miss) Y.M. Freitas, head of the department of micro-

biology, explains that it is customary for members of the staff to worry over the performance of students as if they were all prize students (as indeed, given the competition for places, they are). But even if it were not for the university ordinance restricting student entry (to about 550 for the first-year degree courses), there would be no room for larger numbers.

Teaching science in these circumstances is a constant headache, but the science departments are proud of the way in which they have fashioned a large laboratory-cum-classroom, perhaps 12m x 8m, for their new life science course. The blackboard earlier this year was decorated with what was obviously part of an explanation of DNA. The cost of experimental work for students is a continuing worry.

So could the central university provide more? The general opinion seems to be that the university has in the past few years shown welcome signs of flexibility. Teachers (at least from this one college) can if they are energetic hope to influence the development of the curriculum. But the university, which specifies in detail the syllabus that degree students must cover, could be more active in helping to put flesh on these bones by providing demonstrations of how this material could most usefully be taught. Access by college teachers for their own research to equipment located centrally is, however, being improved, while there is a general welcome for the proposal — not yet a fact — that within the University of Bombay the first of the three annual examinations required

for a first degree should be left to the colleges. (The task of administering these examinations centrally is gigantic at all the federal universities, some of which recruit part-time helpers for the purpose.)

A greater measure of autonomy would probably not be welcome, at least in such a small institution, if only because the faculty, fewer than a hundred covering arts and science, is hardly large enough to pretend to be a university faculty. But at least some members of the teaching staff, many of whom have been able to keep alive their research interests in these difficult circumstances, resent being paid less (Rs600 a month) than members of the university proper. (They also complain of the fly-by-night colleges within the university, where shoestring budgets mean that teachers are paid even less.)

Proof that St Xavier's is far from fly-by-night is to be found in the Blatter Herbarium, by common consent the largest collection of the plants of north-west India, begun in the nineteenth century by Father Ethelbert Blatter, a Swiss Jesuit who was principal of the college from 1919-24. The herbarium is now reached by climbing the steps in one of the wedding-cake turrets. It consists, as such collections do, of pressed dried flowers and plants in folders that can be retrieved by reference to a card index. (Mrs Saramma Almeida, who looks after the collection, does not think it will be possible soon to obtain a micro-processor to keep the catalogue.) But the collection has been and no doubt will still be the focus for continuing research — something else by which to be distinguished among the affiliated colleges. □

"students from the colleges these days are no good". Their preparation in physics and mathematics, he says, is second rate, with the result that the good students opt for management and business studies "where anyway the salaries are better". He regrets the lack of opportunity for teaching undergraduates, or for influencing their teaching.

"What nonsense!" says his vice-chancellor, Dr Gurbakhsh Singh, who goes on to say that the academics at the central university are well placed to determine the curriculum and who would almost certainly be welcome if they chose to spend time teaching at the colleges. Reality is not quite so simple, given the jealousy with which the colleges guard their autonomy. The disagreement rather reflects the common academic view that the teaching of undergraduates is a demeaning occupation. (College teachers are, in any case, paid even less than those employed centrally, and are less likely to enjoy the gift of a subsidized bungalow on the campus grounds.)

The colleges affiliated within the ambit of federal universities are usually a mixed bag. Some, perhaps early nineteenth century missionary foundations (see above), predate the universities to which

they belong. Others may be much more recent, perhaps founded and for practical purposes managed by industrial companies which have calculated that there are political or even financial benefits to be won by demonstrating to a state government a willingness to help the provision of higher education.

Students embarking on higher education are thus able to choose from a diversity of undergraduate colleges. Entry to the "better" colleges is difficult, especially now that many universities (that of Bombay for example) specify the numbers of students that colleges may take in. At the other end of the spectrum, the eye of the needle is wide enough to accommodate most of those who wish to pass through. Often, local politicians and other influential people are able to subvert prescribed entry regulations in the interests of their proteges.

The quality of a college education, even within a federal university, is in the circumstances patchy. The capacity of a university administration to influence and to improve will often be limited not only by its preoccupation with other matters but by the degree to which individual colleges enjoy a measure of financial independence as well as by more familiar constraints, shortages

of people and of resources for example. At many universities, students may register for a degree and, three years later, take the appropriate examinations, without in the meantime having signed on with a college.

The general complaint is that college education consists largely of rote learning, the more particular complaint that learning by heart is practised within a curriculum which is out of date, inflexible and undemanding. The knowledge and intellectual capacity required of students graduating from run-of-the-mill universities in India exceeds that of 18-year-olds leaving British high schools with qualifications in specialized "Advanced" or A-level examinations, but not by much. The reputations of the best colleges stem from their capacity to provide a much better education.

This inhomogeneity is one of the reasons why some universities are cautiously allowing their stronger affiliated colleges a measure of autonomy, over the curriculum for example, within the university as a whole. The University of Madurai, in the far south, now has four colleges linked with the university in this way. For the distant future, these institutions are potentially universities in their own right. But that prospect is still a very long way off.

Meanwhile, the college system remains the Achilles heel of the university system, the means by which standards are determined by those of the weakest. The social and political pressures ensure that degrees, necessary qualifications for senior government appointments, cannot be withheld *en masse*. But the colleges are also the principal seats of political unrest within the universities.

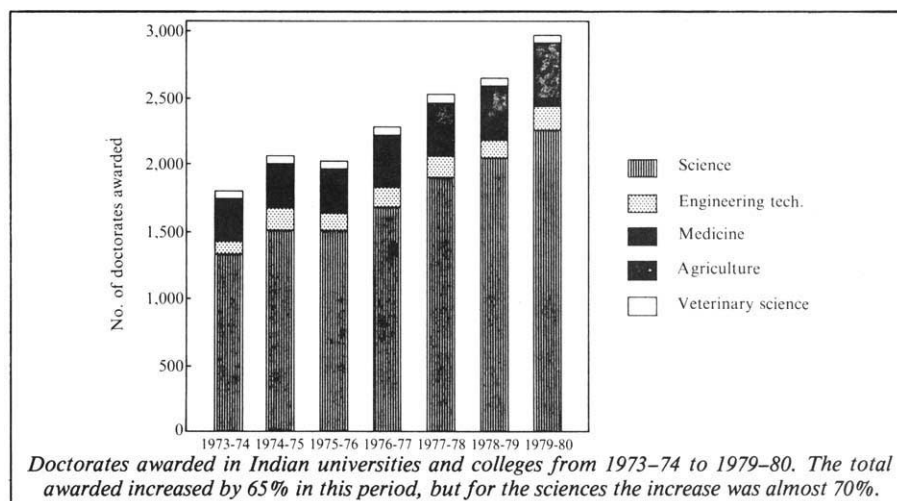
By comparison, the graduate schools of the federal universities are comparatively serene (although this is not the case at exclusively graduate universities such as the Jawaharlal Nehru University at Delhi, repeatedly closed either by students or the authorities in the past few years). Two-year MSc courses yield qualifications necessary for more elevated government appointments and themselves feed into three-year PhD courses. Broadly speaking, the students function as the arms and legs of university research.

The size of these central departments can be huge by standards established elsewhere. The botany department at the University of Delhi, for example, consists of more than 200 people, more than half of them graduate students. The result is a sense of scholarly mass-production.

Not all the universities are like this. Madurai-Kamaraj University in Tamil Nadu (the huge state of which Madras is the capital) was separated from the University of Madras less than twenty years ago, taking under its wing roughly 100 colleges previously affiliated to Madras. Like many other Indian universities, but more successfully, Madurai runs a correspondence course college for those unable to be full-time students.

The university's chief claim on public attention is, however, its school of biological sciences, widely spoken of throughout India as an up and coming place. The secret seems to be a relatively young faculty, many of whose members are conscious of the need that their work should stand comparison with what is being done elsewhere, a tradition that senior scholars discuss their work with each other and the willingness of these same people to boast about their junior colleague's work. How long, one wonders, will the Grand Old Man syndrome, every full professor's belief in himself as some kind of fountainhead of wisdom, be kept at bay?

This one department shows how successfully it is possible for a well-run department to make use of the government's generous support of scientific research through, for example, the Department of Science and Technology. So why are not all departments like the Department of Biological Sciences at Madurai? Much depends on the people, much on the local bureaucracy. One academic complains that even when he has won a grant to carry out some piece of research, he then has to negotiate with the university administration for "permission to spend Rs100" from the money sitting in the university's



bank account.

Sheer size is often the excuse for the obtrusiveness of the administration, but even small places are not always free from the paperwork peril. Size is also, of course, the enemy of quality, which is why there is growing interest among Indian academics in smaller institutions in which undergraduates and graduate teaching live side by side. There are three groups of institutions of this kind, typified by the five Indian Institutes of Technology (set up in the 1960s with financial support from overseas governments), the Indian Institute of Science at Bangalore (a distinctive institution dating from 1909) and those among the regular universities — the Jadavpur University in Calcutta, for example.

The institutes of technology (known everywhere as the IITs) are frankly intended as the Indian equivalent of the Massachusetts Institute of Technology (MIT). By all Indian standards they are enormously successful institutions. Student entry is by a national competitive examination among 80,000 high-school students every year. Success requires that a student should have spent 12 years in school (on the pattern now known as "10 plus 2", analogous to the English system in which two years of "advanced level" teaching follow the general high-school course). Student entry is strictly limited, courses are almost exclusively technological (but there is talk at some of these institutions of a leavening of humanities or social science) and the universities are both attractive and well equipped.

The snag is that a large proportion of those who graduate promptly emigrate, at least for a time. Meaningful figures are not easily come by, principally because there is no way of knowing how many of those who move overseas after graduation return with still better qualifications. But many in India shake their heads over what is happening, and agree with the academic who says that the "IITs are India's most generous gift to the United States".

The Indian Institute of Science at Bangalore, originally a Tata foundation (like the Tata Institute of Fundamental Research at Bombay) is both a research

institute and a university. The student body numbers just over 1,000, of whom more than 80 per cent are graduate students. As at the IITs, the educational programme is largely technological, with the handful of undergraduates following courses leading to a degree in engineering. The complaint that graduates leave for overseas once the ink is dry on their degree certificates is almost non-existent, perhaps because those concerned have been given not merely a saleable education but a degree of engagement with the practice of science and technology in India.

Bangalore is widely admired, not least because of the numbers of its graduates who now occupy senior positions elsewhere in Indian academic life. The small scale of the institute makes it manageable, while the generosity of support from the University Grants Commission (more than Rs50 million a year) and from the grant-making agencies makes it possible to sustain a research programme of high quality. The institution seems more like California Institute of Technology than MIT.

Bangalore has also been fortunate in its directors. The present director (due to retire in the next year or so), Professor Sivaraj Ramaseshan, is a perceptive physicist with a background in crystallography (with Professor Dorothy Hodgkin at Oxford). Ramaseshan, now also president of the Bangalore-based Indian Academy of Sciences, is widely respected for his zealous defence of academic standards at Bangalore over the past decade. By his colleagues' account, his success stems largely from the encouragement he provides for heads of department and less exalted academics at the institute, in sharp contrast with the habit of most of those with administrative responsibility of reminding their colleagues of their subordinate status.

Even so, Bangalore has not entirely escaped the creeping bureaucracy. One senior academic at the institute, who returned last year to India after fourteen years in North America, says that he had resolved at the outset to keep his mornings free for research and to set aside the afternoons for administration. In the event, he has found that administration accounts for

90 per cent of his time, and that he has no choice but to spend the morning sitting in the offices of the administrators: "they aren't there in the afternoons". Will this be another case of a returned emigrant whose enthusiastic patriotism is dashed by the system, and who stays only briefly?

The universities in the strict sense still within the system but which have resisted the pressures for growth resemble the other institutions of high quality that stand out like sea-mounts from the general background. Jadavpur University (Calcutta) has its roots in the upswing of Bengali nationalism in the 1900s, and in the formation of the National (or Bengali) Council of Education. Towards the end of British rule, the nascent university was a thorn in the side of the authorities. Now it has fewer than 4,000 students, a campus looking like a university campus and with a substantial amount of student accommodation and a teaching programme (graduate as well as undergraduate) slanted towards science and technology. The Liverpool-trained vice-chancellor, Dr Monindramohan Chakrabarty, is determined to keep the university small, claiming the support of the Government of Bengal to this end.

The success and failures of the Indian university system point clearly to the underlying dilemma. In education and research, small institutions can succeed, fully justifying the widespread conviction in India as elsewhere that the institution of the university can be both a source of skilled people and a humane influence in society. If the pursuit of quality were undivided, it would be clear how the system would develop. But the demand for higher education is insatiable, politically irresistible and, in any case, socially equitable and necessary.

The central government seems aware of the problems, although it may not fully appreciate their consequences. Attempts are being made, by the UGC and other agencies, to discriminate in favour of the better institutions, but political interests, especially those of state governments, work in the direction of even-handedness. Nepotism, both in the selection of students and their graduation and in the appointment of teachers is known to be rife, yet if politicians are able to be appointed even to the staffs of the seven central universities (see p. 595), the chance of rooting them out from the other universities must be small.

The central government's capacity to shape the system is constrained by the circumstance that state governments (and students by their payment of fees) meet the running costs of all but the central universities. Even the best institutions could be ruined overnight by a feckless decision of a regional education minister. And the openness of the Indian political system makes it hard to see how there could be an understanding between the central government and the states about the universities where controlled growth would be encouraged over a period of years ahead. □

Technology institutes

Single-minded exceptions to rule

THE simplest way of recognizing that a university campus is that of one of the five Indian Institutes of Technology (IITs) is to look for several acres of manicured lawns and a group of buildings characteristic of academic architecture of the 1950s and 1960s — post-Corbusier concrete, glass and occasional murals. The Delhi campus is notable for the rock outcrops sticking up from the vast lawns, that at Bombay for the canopied walkways that protect people from the monsoon.

Academically, the five institutes are distinguished by their selectiveness and by their four-year undergraduate courses. Their existence stems from a report to the Government of India by the Sarkar committee in the 1950s, which argued the need for engineering institutions along the lines of those in the United States, mainland Europe and the Soviet Union. At the outset, overseas governments played an important part in the foundation of the institutions. The United States helped to launch the IIT at Kanpur (Uttar Pradesh), Britain that at Delhi, West Germany that at Madras. Kharagpur (West Bengal) is an indigenous institution and that at Bombay owes its origins to help from Unesco and the Soviet Union. As Indian universities go, the IITs are small, mostly with fewer than 3,000 students (of whom a third will typically be graduate students).

Professor A.K. De, still last month the director of the Bombay IIT, attributes the success of his and comparable institutions to the scale on which they are financed. The annual budget exceeds Rs70 million, more than 80 per cent of it direct from the Ministry of Education and Culture (not the University Grants Commission). Research and contract income is a growing proportion of the total. Student fees are small

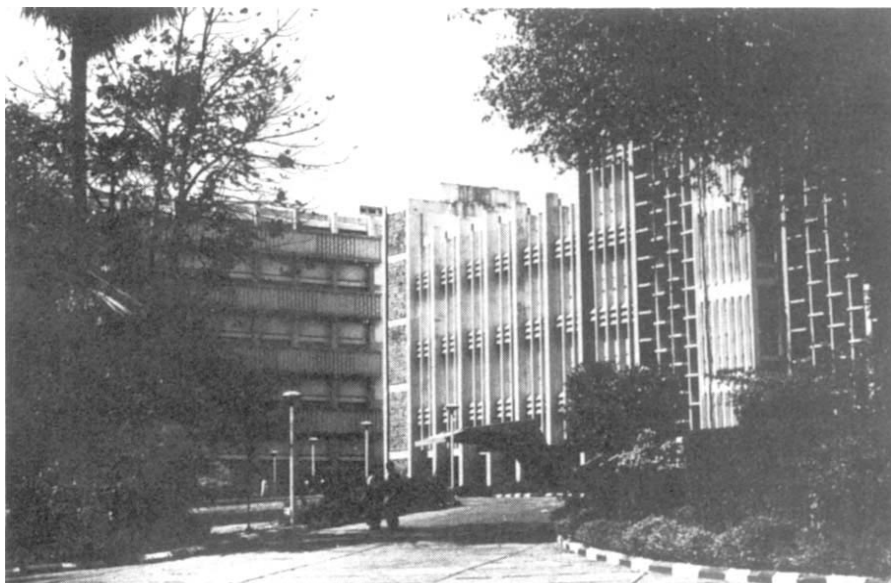
and contribute negligibly to the university's budget.

De emphasizes the importance of the autonomy of the IITs, which are free from interference from both state governments and from the centre (except in respect of student entry). The IITs nevertheless collaborate with each other in the provision of a national examination for those who have spent the two "extra" years at high school. (At Bombay, they hope to phase out the last of the students recruited after only 10 years of school education by 1985.)

Autonomy (and reputation) give the institutions the opportunity to select members of staff competitively, and by methods not very different from those used in the West. Potential members of staff are invited to give a seminar, and decisions about new appointments are discussed among the existing faculty.

The undergraduate curriculum is leavened (to the tune of 8 per cent in time spent) by humanities and the social sciences, while basic science takes up 32 per cent of the full four-year course. Workshop training is an essential part of everybody's course, but Bombay has set its face against "sandwich" courses in which students intercalate a year at work with regular academic work. (The engineering profession in India requires that a graduate should complete 18 months of work training before becoming qualified.)

Bombay offers the traditional engineering courses, but has found in the past few years that computer science is in the greatest demand. The institute operates a computer centre and at the end of last financial year was negotiating with the Government of India for the funds to purchase a Cyber machine — because the telephone system cannot handle data



IIT's concrete and glass.

reliably, the IIT can use the Cray machine at the Tata Institute of Fundamental Research only by having its staff fight with the bullock carts and overloaded trucks along the 25 miles of highway to the city.

By De's account, the success of the IITs is measured by the way in which their graduates are able to command jobs before they graduate. "Every student has a job, some of them have two or three lined up." On the contentious question whether the IITs are a device for exporting India's talent, De estimates that 20 per cent or so of graduating students go abroad immediately, but that the proportion may be as great as 80 per cent among computer science graduates.

Overseas links in the other direction are less conspicuous, at least at Bombay. The capital and equipment used in the foundation of the Bombay IIT came almost entirely from the Soviet Union, both directly and through Unesco. But the Soviet influence is not now apparent, according to De. (The "British" IIT at Delhi has, however, been diligent in forging newer links with European institutions in France, Switzerland and West Germany.)

Nor is Bombay keen on overseas evaluation of its educational programmes. External evaluation of courses would be expensive and, in any case, "we want to know what the students have learned, not what they know". De emphasizes the importance of the flexibility that derives from the autonomy of the IITs, saying that his faculty can change what it teaches

quickly, and so keep up with the development of engineering techniques elsewhere.

The self-confidence of the IITs seems formidably recognized in their growing links with Indian industry. De says that industrial companies began to recognize their importance in the 1970s, with the result that the Bombay institute now earns some Rs8 million a year from industrial research and development contracts. As elsewhere, staff at the institute are encouraged to take on industrial consultancies, and are allowed to keep 70 per cent of what they earn within a limit of roughly one year's salary.

So, if the IITs are a big success, why not have more of them? As things are, they produce fewer than 2,000 graduate engineers a year between them. De considers this to be a question of resources. In previous decades, he says, the government "has poured money" into the development of national laboratories such as those operated by the Council of Scientific and Industrial Research. Investment in institutions such as the IITs might have been wiser.

And what can specialized institutions such as these offer India as a whole? In the long run, De says, their crucial function is to help redress the balance of the import-export trade, still weighted against India. And even the problems of development in India may in the end require high-technology solutions, which is why De welcomes the grant from the State Bank of India for the establishment of a chair in development studies. □

cluded Castes and Tribes but for the sons and daughters of their own employees.

Especially where the universities operate high schools of their own, the benefit accrues mostly to "children of the university's own staff and their dependants" who then "pedal their way up to postgraduate courses, research programmes and then on to teaching posts". The admission of students "at the discretion of the vice-chancellor" is a dangerous source of external influence on universities, while the failure of the Jawaharlal Nehru University to check on "bogus" certificates of performance typifies another weakness. The inquiry pleads that universities should determine their student capacity objectively and stick by the targets thus arrived at.

The charge of academic inbreeding is sustained by statistics for staff recruitment at the central universities. Between 1975 and 1981 for example, 75 of 80 academics appointed professor at the Banaras Hindu University were internal candidates, as were 90 per cent of the 177 readers appointed during the same period. At the same university, 73 per cent of newly appointed lecturers during this period were from within the university, where they had been either research fellows or teachers employed on a temporary basis, whose qualifications do not have to meet the standards promulgated by UGC. The inquiry deplores reports that heads of departments have often made decisions about new academic appointments before the statutory selection committees have deliberated.

The inquiry gathers some disturbing evidence of the work practice of academics. The UGC scheme whereby up to 10 per cent of college teachers may have three years' leave on full pay seems widely abused: in the evidence by one college principal (unnamed and now retired):

"The leave is availed of but no degree or no concrete work done in the period is produced; and there is nobody to call the defaulters to account. There are several cases in my college of 3 years leave taken and no work done . . . it practically means a 3-year paid holiday. Research has become a cloak for many malpractices and much waste of public money . . ."

The inquiry notes that even at the University of Delhi, right under its own nose, "repeated requests" for information on the operation of the Faculty Improvement Programme have yielded nothing.

The work-load of a college teacher seems not especially onerous. On one calculation, a college teacher may work for no more than 64 days a year after allowance for entitlement to days off and for the disruption caused by strikes. Yet the inquiry endorses the complaints of some of its witnesses that "teachers come for their lectures and rush away after them so that there is no opportunity for students to meet them . . . after 1.00 p.m. the college is like a graveyard".

This vacuum is a fertile breeding-ground for violence. The report of the inquiry has several graphic accounts of student

Central universities

Breeding ground for violence?

NEPOTISM, disorder (even violence) and maladministration are rife even in the seven universities supported directly by the Government of India. This is the sad conclusion of the committee of inquiry into the working of the central universities, which reported at the beginning of this year. One measure of the importance of the inquiry is that the committee's chairman was Mrs Madhuri Shah, the chairman of the University Grants Commission (UGC) which administers the budgets of the seven universities.

The central universities are intended to be national institutions. Three of them — the University of Delhi, the Aligarh Muslim University and the Banaras Hindu University — acquired that status before independence. Visva-Bharati, an institution for music and the arts, was recognized as of national importance in 1951. The Jawaharlal Nehru University was founded as a post-graduate institution in 1969, the North-Eastern Hill University in 1973 (to meet the needs of the small states in the north-east of India) and the University of Hyderabad in 1974 with the objective of encouraging interdisciplinary teaching and research.

The problems with which the inquiry was concerned are typified by the statement in the report that a visit to the University of Delhi planned for 7 October 1982 could not take place "in view of the strike in the university", that statistical information on the numbers of teachers in post could not be supplied by some of the universities and that its attempt to discover what the government had done about the recommendations of an earlier comparable committee in 1968 was met with the reply that "despite best efforts . . . we have not been able to locate the relevant file . . . but our efforts are continuing".

With the exception of the Jawaharlal Nehru University (in Delhi), none of the universities seems to be functioning as a national institution. The practice of the Aligarh Muslim University of recruiting 80 per cent of its students from the state (Uttar Pradesh) in which it is located is typical of what happens at other central universities. The committee of inquiry is constantly uneasy about the practice of most universities of reserving up to 20 per cent of the available places not merely for the socially disadvantaged groups known as the Sche-

troubles sparked off by attempts to discipline individual students, accusations of sexual harassment against teachers and, commonly, about hostel accommodation. The result — “the agony experienced by these universities is unmeasurable, and it is destroying the fabric of the system”.

The special Indian student technique of intimidation is the *gherao*. A crowd will occupy the office or house of a professor or vice-chancellor, crowd him into a corner and shout abuse at him for hours or even days on end. In March last year, the vice-chancellor, rector and acting registrar of the Jawaharlal Nehru University were corraled for 48 hours and “could not eat or sleep” until the police were called, the ring-leaders arrested and their fellows provoked to a “rampage” after which the university was closed for several months.

The causes of these disputes, the inquiry says, lie in the failure of the universities to determine who shall be entitled to live in student hostels, with the result that scarce lodging space is used by people without substantial connections with the academic institution but who often have a vested interest in agitation, the lack of a corporate sense of an academic community, the dilatoriness of administrations in dealing with grievances of individual students and the neglect by academics of their responsibilities for teaching and discipline.

The inquiry does not endorse the common view that student troubles would go away if only the universities could be rid of politics and politicians. Liberally, it says that it is inevitable that a “community of

thinking people” should be politically engaged. But the inquiry finds that the political activity which it has witnessed on the campuses of the central universities “is of a degenerate nature which is a blot on the concept of politics” and may be held to be the “politics of expediency”.

On the involvement of the political parties on university campuses, the inquiry deplores the use of party organizations to prevent disciplinary action by university authorities, for example against students who may have “copied in the examinations”, and the “politics of corruption” by means of which money is used to buy votes or to harass the authorities.

The inquiry also notes that academics are not above using their political connections to further their own ends, as when they have a financial interest in a purchase to be made by a university or are aggrieved that they have not been appointed to some post. The deliberate use of groups of agitators to provoke confrontations with universities and the police is said to be a common way of prolonging university disputes.

The inquiry’s remedy for this state of affairs is hortatory — let the “mature” political parties exercise restraint, not seek to provide political support for groups within a university who may be working against a vice-chancellor or the administration and let government ministers not seek university posts on their own account. The principle is admirable: but will the exhortation be heeded by those to whom it is addressed? □

system it oversees. The chief instrument is the system where university departments can be designated as centres of advanced study for periods of fifteen years at a time, and given extra funds for staff and research support. The hope is that at least a proportion of these will become so much admired nationally that they will attract academics from elsewhere in spite of the principal obstacles to academic mobility — housing and schooling — and so be able to practise what UGC is always preaching at them — the selection of academic staff on merit from the national pool of talent. The scheme has been operating for a decade now, but began only tentatively, so that it may be too soon for evaluation.

This observer’s impression is that the scheme has backed losers as well as winners. The chosen departments, hitherto exclusively in science, are proud of their distinction but some at least seem to have rested on these now ancient laurels. Larger resources spent on even fewer centres might be a good investment.

More recently, UGC has set out to improve the availability of modern equipment by setting up 54 regional equipment centres (such as that at the Bose Institute in Calcutta, see p.590) to which academics from a wide area may have access. The scheme seems to work reasonably well (even though at least one of these centres still seems to have a good deal of its equipment in packing cases). Users of the equipment (or their universities) pay fees on a scale that would excite the envy of investigators elsewhere. The plan is to set up computer centres (there are three already) along similar lines, but Mrs Shah is anxious to point out that “the sky is not the limit”.

For the encouragement of people, UGC is now also fostering a National Talent Scheme to provide postdoctoral support for mathematicians and scientists. There are about 100 fellows now; Mrs Shah would like to see 500. At the other end of the age spectrum, there is a new scheme for giving up to 100 academics the distinction of “professor of eminence” and an extra Rs500 a year (roughly a third of the cost of a *Nature* subscription) to go with it. In the hope to tempting back Indian emigres, for a year or more, UGC offers stipends of Rs3,000 a year to visiting professors, who will probably be provided with housing and the costs of air travel by the universities concerned.

On the organization of universities, Mrs Shah would indeed like to see closer links between university departments and the government laboratories scattered over India. She is pushing for autonomy for more of the constituent colleges of the federal universities as well as for an understanding that vice-chancellors should be appointed for fixed terms of five years (so that they will not become the hostages of local politicians when canvassing for a second term). She says she will require that heads of universities should not in future be members of the committees appointing less senior

Grants Commission

Hoping for consolidation

DR Madhuri Shah, the first women chairman of India’s University Grants Commission (UGC), has three outstanding qualifications — a background in the Bombay educational system, an unaggressive directness that wins the respect even of her critics and the personal support of the Prime Minister, Mrs Indira Gandhi. All three are important but the last is essential.

She is ready to acknowledge that the Indian universities are “a problem” but is unwilling to use more emphatic words. She asks that visitors should also understand that the “hopes and aspirations” of the people for higher education cannot indefinitely be denied.

Constitutionally, UGC, modelled on what used to be the comparable organization in the United Kingdom, is meant as a buffer between the central government and the universities. While the chief conduit for central government spending on the universities, UGC is emphatically not a part of the government. But equally, and in contrast with the analogous organization in Britain, UGC has no financial control over individual universities, with the exception of the seven central universities (see above).

The result is that it must function by the exercise of its statutory powers (which are to certify that institutions are worthy of university status), by exhortation (of central government as well as of the system) and by using its discretionary funds to provide incentives. UGC has carrots but no sticks of its own.

Mrs Shah’s hopes for the years ahead turn chiefly around the way in which the growth of the university system has recently slackened. For most of the period since independence, the rate of growth has been 13.5 per cent a year, but in the past five years the rate has been merely 3.5 per cent. This provides a breathing space.

But even so, qualitative change continues. Universities are being compelled to redesign courses under the influence of “feedback from the employment market”, perhaps explaining why some once-strong departments are less able than in the past to recruit talented students. Mrs Shah agrees that the physics department at the University of Delhi, for example, may be suffering in this way.

Meanwhile, UGC is seeking to capitalize on what is already excellent within the

members of their staffs, but there is some doubt whether this can be enforced.

One of Mrs Shah's concerns is that there should be better coordination between UGC and the Department of Science and Technology, if only so that the two organizations can work together in the selection of centres of excellence. She will be "pressing" for UGC representation.

Meanwhile, UGC seems not to be hamstrung for the odd crore or two of rupees. One evening, the vice-chancellor of the

University of Delhi confided that he had been a little startled to hear just a few days earlier that UGC had given him an extra Rs30 million, two-thirds of it for building faculty housing. Mrs Shah explains that she has ingeniously set up a kind of finance corporation from which housing funds can be borrowed and repaid out of the rents that academics pay. The other Rs10 million is no doubt a measure of the agreement she has won from the central government that the universities do deserve support. □

Food science

Diet for seven-hundred million

If Hindus avoid beef and Moslems pork, there is always mutton? Not necessarily, in a country where a large proportion of the population is vegetarian.

These are some of the reasons why food research in India, like many other fields of research, must tackle problems of its own. And this is partly why food research — post-harvest technology — has prospered in the wake of the Green Revolution. Another is the importance of many crops, from cashew kernels to natural oils — in India's export trade.

The Council for Scientific and Industrial Research (CSIR) is one of the chief sponsors of this research, through three of its regional research laboratories (at Jammu, Hyderabad and Trivandrum). There are also specialized centres such as the Tea Research Association (Calcutta) and the National Dairy Research Institute. The chief focus of much of this research is the CSIR Central Food Technological Research Institute (CFTRI) in Mysore.

Established in 1950 in a former palace, CFTRI is probably now the largest institute of its kind in the world. Together with research, it takes part in international development and training programmes (flour-milling with Switzerland, abattoir technology with Denmark), and is also a kind of extension service for food technology. Thus it runs a national information service, publishes a monthly abstract bulletin, a bimonthly newsletter (in English, Hindi and Kannada) and professional journals such as *Indian Food Industry* and the *Journal of Food Science and Technology*, and a series of free brochures and booklets on new developments. It takes on sponsored research for industry (on a non-exclusive basis) and has out-stations at Ludhiana, Lucknow, Nagpur, Bombay, Hyderabad and Mangalore. It now has a staff of about 900 including some 250 scientists and 350 technicians, and an annual expenditure of over Rs20 million.

The first aim of CFTRI's charter is the "maximum utilization of available scientific and technological knowledge with necessary modifications to suit our socioeconomic needs". Much research at CFTRI is, indeed, conditioned by India's

specific dietary customs and problems.

It was long believed, for example, that malnutrition in India was associated with the vegetarian diet of strict Hinduism and was due, first and foremost, to a lack of protein. But it is now established that the protein content of an Indian vegetarian diet (in the form of milk products and pulses) is more than sufficient — provided that the family budget can run to it. The main problem of India's poor is not a lack of protein, but of all types of nutrients, including carbohydrates. Indeed, since strictly observant Hindus come mostly from formerly high-caste and therefore prosperous families, malnutrition may even be less common among vegetarians. So CFTRI's work on food supplements for use in emergency feeding programmes includes not only protein supplements (such as "chewy candy" with a 12 or 16 per cent content of vegetable protein) but also a low-cost high-energy cereal food, prepared from a mixture of local grains and pulses, whose composition can be varied to suit local availability and tastes.

Even among non-vegetarians, religious sensitivities can cause problems. The Hindu avoidance of beef and the Moslem prohibition on pork has prompted the development of production techniques for mutton-based products such as sausages and corned meat. A recent development

has been the isolation of a fraction from the crude protease of *Rhizopus oligosporus* which has properties close to those of animal rennet for milk-clotting and cheese-ripening. This, it is hoped, will ultimately replace the imported animal rennet at present used by the dairy industry — and which even strict Hindus are, apparently, willing to tolerate since it is not produced within India.

Here as elsewhere the effort to reduce dependence on imports is a recurrent theme. CFTRI is proud of its formulation of an infant food based on buffalo milk, using a special technology to reduce the butter-fat content to an acceptable 14-15 per cent. This has given rise to a major indigenous industry, and yields a valuable by-product (the excess butter fat) which helps to keep down the price of the finished product. Similarly, since India does not produce sufficient malting barley for its brewing industry, a major CFTRI project has been the investigation of the possibility of making beer from low-grade cereals such as *ragi* (*Elusine coracana*), a millet.

Special attention is paid to the avoidance of waste. A technique for removing the acrid and astringent principles from cashew apple, a by-product of the cashew nut industry, allows its use as a basis for soft drinks, jams, pickles and curry. In Goa, cashew apple is used to make a traditional liquor, *fenny*, for which CFTRI has developed new technology. Special attention is paid to food protection and preservation during storage. Pesticides and fumigants for stored cereals, suitable for use by farmers with little or no formal education and whose families live and sleep in close proximity to the grain store, have been developed, as have cheap racks for the transport of fruit by rail with minimum spoilage.

CFTRI claims to be the first institution to point out the dangers of using DDT in conjunction with food grains. Work is now being carried out on the development of ecologically safe grain protectants, such as tricalcium phosphate which destroys grain-infesting insects but which is non-toxic to



CFTRI's headquarters — formerly a dowager princess's palace and now the centre of perhaps the world's largest food research organization.

humans and the environment.

Finally, while serving the home market, CFTRI has a wide programme of export-related research aimed at the affluent world. New variants of traditional products (instant teas and coffees, flavoured teas) have been developed. For the spice industry, rats are being used to assess harmful side effects at high doses and to define standard specifications. (In the mid-1970s, CFTRI determined that turmeric, which makes curry dishes yellow, is harmless even at 100 times the normal curry-

eater's intake, thereby allaying an international wave of alarm about the spice.)

An important project is the extracting of oleoresins and oils from India's traditional spice products. This has proved a major earner of foreign exchange (Rs 76.5 million in 1980-81) but also has emotive overtones. It was the trade in raw spices that first drew the Europeans to India. By exporting not raw spices but a finished product of high value, India's food technologists like to think they have helped to shake off the shackles of a colonial past. **Vera Rich**

Science for schools

Making an exhibition of itself

INDIA's "Method of Science" exhibition, first planned in 1975, will probably not now be opened until after the general election expected within the year. The exhibition, intended for senior secondary school students, was planned as a permanent display, presenting science not as a collection of achievements and gadgets but as an intellectual discipline based on deductive reasoning and inference. It was assembled in New Delhi in 1977, dismantled overnight by government order in August 1978, reassembled in Hyderabad in 1983 but is still, at the time of writing, not open to the public.

The exhibition was originally proposed by Dr Rais Ahmed, the then director of the National Council of Educational Research and Training (NCERT) and designed by Dr Pushpa Bhargava, director of the Hyderabad Centre for Cellular and Molecular Biology (CCMB). The completed design was opposed by moral reformers, leaders of religious cults, homoeopaths, civil servants, accountants, politicians and even some members of the scientific establishment who, with the change of government in March 1977, tempered their original enthusiasm to the prevailing official line. The Indian Humanist Society, however, took legal action on behalf of the exhibition, and the issue was taken up in the standing committee on the Safeguard of the Pursuit of Science of the International Council of Scientific Unions.

Most overt criticism has been detailed. The financial lobby questioned Bhargava's spending on visual material ("Could not the captions have been written with chalk on blackboards?"). Another controversy arose over a display allowing visitors to decide whether propositions are true, false or not yet determined. A wraith-like figure, identifiable as female only by its floating hair, which simultaneously leaves and enters a door is used to illustrate the concept of motion faster than light, but was condemned by some as a lascivious nude. Others objected to the inclusion of Marx, Engels and Lenin in the series of "21 landmarks in the history of the method of science".

An exhibit entitled "Science has no high priests who cannot be questioned" con-

trasted a panel showing a young scientist at a conference questioning the results of an eminent lecturer with a "godman" surrounded by a group of humble disciples. To avoid accusations of caricature, the face of the "godman" was a portrait of one of the organizers, but controversy sprang up on the ground that the gold watch in his hand might remind viewers that materializing watches is part of the repertoire of a particular "godman" with a large following. (The artist obligingly replaced the watch by a ring, but the critics were still not satisfied.)

The more serious objections have never been made explicit. The exhibition questions the value of homoeopathic medicine, without which the Indian health services would not function. It also questions astrology, while several leading politicians are known to order their professional lives according to the stars. Perhaps most dangerous of all, to the Janata Government of 1977-79, it is anti-authoritarian. It teaches nothing and asks visitors to accept nothing on trust, even the message of the exhibition itself.

Official support for the exhibition lapsed with the defeat of Mrs Indira Gandhi's government in 1977. Ironically, it might well have opened in Delhi if Dr Rais Ahmed and the Education Minister, Professor Nurul Hasan, had not pressed for Mrs Gandhi herself to inaugurate the exhibition. The approach of the elections, however, meant that Mrs Gandhi had no convenient slot in her diary until too late.

Recently, political events have once more conspired to delay the opening. The Hyderabad authorities, who have given their backing to the reassembled exhibition, felt that it would be proper for the opening ceremony to be performed by Professor Nurul Hasan, who is now Indian Ambassador to the Soviet Union. Plans for him to open the exhibition in March were disrupted by the death of Mr Yuri Andropov. The inauguration is now tentatively scheduled for November. While the exhibition itself stresses the anti-authoritarian nature of science, those responsible for it seem unwilling to do without the blessing of a political guru. **Vera Rich**

Green revolution

Caring for a continent

INDIA is two countries, one mostly urban and partly middle-class, the other rural and mostly poor. And on this simplified model, the interactions between the two countries are rudimentary.

Conspicuous though the city slums may be to visitors, the problem that matters most is that of the rural poor, almost all dependent on the land or on those who own land. This is where illiteracy is acknowledged to be a third (and may be twice as much), where infection rules (as goitre is endemic along the northern foothills) and where the rate of population growth is highest. What will the other India do for this people?

One thing has happened already: the techniques of farming even in the rural areas have improved to the point at which they (and India) feed themselves. The green revolution has come and will not now go away. The big change came between 1965 and 1977, when the production of wheat increased threefold, to more than 30 million tonnes a year. The rice harvest increased by two-thirds in the same time, coarse grains by one-third.

The revolution was more than a century late. The first impetus for agricultural improvement in India came after the American Civil War, when British cotton manufacturers demanded (without success) that the administration should make India into a better source of raw material.

Later famines persuaded Indian administrators to tentative steps towards improvement, but only the aftermath of the disastrous famine of 1899 persuaded Lord Curzon (Viceroy soon afterwards) to set up the Imperial Agricultural Research Institute (at Pusa in 1905). Its direct descendant is the institute now at Delhi (with "Imperial" changed to "Indian").

The Council of Agricultural Research founded in 1929 was the direct antecedent of the organization now in being. By then the British administration and the provincial government, concerned not least of all about their revenues, has seen the importance of on-the-ground advice for farmers. After independence, the example of the land-grant colleges in the United States was acknowledged to be relevant, and the union government was hard pressed (by the states) to set up specialized agricultural universities for research and for training the people who would man the extension services. By 1960, the first of 23 agricultural universities had been founded. This, a decade later, was the foundation on which the green revolution was built.

Dr Maharaj Singh, now deputy director-general for research at the Indian Council

of Agricultural Research, was director of research at the agricultural university at Pantnagar. He tells a tale of heady success during the 1960s. The 10,500 acres that the university had inherited from the previously extant state-run agricultural research station were mostly converted into a seed farm for propagating the newly introduced strains of wheat. At the height of the green revolution in the late 1960s, the seed farm produced 180,000 quintals (1 quintal equals 100 kg) of seed, and was able to sell this to local farmers at a profit of Rs15 million.

Part of the secret of success, Singh says, is that the university was so closely in touch with the farmers (who were able to buy simply enough seed for their farms) that its staff could take steps to see that those who bought the new seed were clear how it should be used. The availability of fertilizers was crucial, the bureaucratic management of the irrigation system at first an impediment.

The agricultural universities function well, Singh says, partly because they are able to respond quickly to market forces — local farmers' needs — and partly because they are small. At Pantnagar, the annual intake of students is 150, who follow four-year courses in veterinary as well as crop science.

While the production of the basic grains can be still further increased (and must be if the population keeps growing), Singh points out that there is scope for this — the penetration of the green revolution is far from complete. But the research council is now also turning its attention to schemes for vastly improving the yield of cash crops such as castor oil and mustard (at an experimental farm in Gujarat), which can be sold abroad for hard currency.

The success of this enterprise owes something to its long traditions. Indian administrations have been worrying about agricultural production for more than a century, while the hard work of setting up and strengthening the extension services long predates the availability of the high-yielding strains. But how has the enterprise escaped the clutches of the bureaucracy? First, it has been helped to keep its sense of direction by the influence of market forces — farmers' needs. Second, it has not escaped scot-free.

The administration of the Indian Agricultural Research Institute was fiercely criticized in November last year by the Indian Supreme Court for its mismanagement of personnel promotion. Two scientists who had complained in 1972 that another had been unfairly appointed to a post for which they had competed were retrospectively ordered to be given the status of which they had been deprived. This bitterly fought case could have arisen only in India.

The success in agriculture is an example to be followed in the provision of health care but only with difficulty. The problems are more complicated — the prevalence of

viral infections, bacterial infections and tropical diseases, endemic diseases with an environmental origin (goitre due to iodine deficiency) and the peculiarly Indian incidence of oral cancer. But organization, the delivery of health care to 700,000 villages for example, is a constant problem. Dr V. Ramalingaswami, now director-general of the Indian Council of Medical Research, explains that the first need is to "make available technologies available" under the forthcoming seventh five-year plan and to "improve existing technology". One essential component of any programme for the years ahead must be to make immunization more effective. Whether the goal of providing 100 per cent protection against bacterial and viral infection by 1990 can be effected remains to be seen.

A paediatrician in Calcutta will not easily be convinced. He says that in his clinic, he could prevent 50,000 cases of serious infection at a cost of \$1,000. He complains that the rate of infant and even maternal mortality at childbirth is intolerably higher than it should be for lack of funds with which to ensure normal hygiene. Where should the \$1,000 come from? State governments are the chief source of funds for the provision of health care on a local basis. They seem often to consider that they have better ways to spend their money.

Meanwhile, medical research is aimed at the improvement of cholera vaccines, where the search for an adjuvant is pre-occupying, and is alarmed about the resurgence of malaria. From time to time, newspapers in India now refer to outbreaks of a "mysterious infection", which is increasingly a euphemism for malaria. Yet in 1967, there were no deaths from malaria in India, while the number of cases of people with malarial parasites in their blood had fallen to half a million. But by 1976, it is estimated, the number of malarial infections of people had increased to 7 million. So was it in retrospect a mistake, in the 1960s, to have given up the use of DDT for the control of mosquitoes? Ramalingaswami says (after a long pause) he is "reasonably certain that malaria would have been eliminated" if the eradication programme had been continued vigorously.

The other serious disease that preoccupies the whole of medical research in India is population growth. Laboratories throughout the country are hunting for novel contraceptives, in Indian plants, by organic synthesis and by the careful study of every aspect of the process of human reproduction. Making medically safe contraceptive devices (intrauterine devices for example) is another preoccupation. But then, people say, in Kerala, the birthrate has fallen to the point at which the growth rate of the population stems only from increased longevity. Kerala is also the only state in which the population is fully literate. "We don't understand what's happening in Kerala." □

Science at grassroots

WHEN an Indian politician addresses a gathering of scientists, he (or she) will almost certainly refer to the application of science to rural development. The precise meaning of this cliché is seldom defined, but it refers not to the immediate needs of agriculture or irrigation, but to the establishment of income-earning industries in the rural areas.

How is the technology for such industries to be developed and, more important, imparted to the often illiterate villagers? And just how much is being done? Last January, when Mrs Gandhi visited Bihar to open the annual National Science Congress, she implied that the state had done little or nothing for its backward areas. Yet the science congress was taking place at the Birla Institute of Technology (BIT), which since 1976 has run a special Small Industries Research, Training and Development Organization (SIRTDO) to sponsor such projects.

SIRTDO is a joint venture of BIT, the Government of Bihar and the Birla Institute of Scientific Research which has grown out of a "technical entrepreneurship programme" — a kind of science park — for young technologists to develop new devices which established industries were reluctant to take up.

The theory is simple. A group of "tribals" (descendants of the indigenous pre-Aryan population and one of the least advanced communities in India) is enrolled in a training course, taught a new technology and also good work habits (regularity) and then sent back to their own villages. To ensure that they have an opportunity to practise their skills, a new Institute for Rural Industrialization (IRI) was set up in 1982. The first two factories set up under the scheme produced hand-tools, but now there are plants producing electronic components as well.

The weakness of all Indian rural industry is dependence on suppliers and on middlemen, which services are largely provided by IRI, which also helps with the initial (generally fairly modest) capital outlay. The factories themselves are organized on a cooperative profit-sharing basis.

According to Dr A.K. Basu, head of the SIRTDO centre at BIT (other centres have now been set up in various towns in Bihar), trainees have a high degree of initiative and responsibility. Within three months, workers in the first factory had expanded their range of hand-tools of their own design. Electronic assembly workers have often proposed improvements in layout, or a more rational method of working.

Some of Dr Basu's anecdotes reflect perhaps an initial enthusiasm that may later wane. He says, however, that he is encouraged that in villages with small factories, agricultural productivity rises significantly, as profits are reinvested in improving family farms. Vera Rich

India in space

Reaching for the stars

LAST week, India's space programme reached another charismatic climax with the visit to the Salyut-7 space station of cosmonaut Rakesh Sharma — the first representative of the non-aligned nations to go into space. But this is only the frosting on a much larger cake.

The Indian space programme, begun with substantial foreign help from the Soviet Union, the United States, France, West Germany and Czechoslovakia, aims at increasing self-sufficiency. Even in the modest technology of high-altitude rockets for cloud seeding, the Birla Institute of Technology (Ranchi) has a programme, supported by the Institute of Agricultural Research, to develop rocket fuels from shellac with the hope that this will ultimately increase the prosperity of the shellac-producing areas of Bihar.

Since its inception in 1961, the Indian space programme has in fact been strongly orientated towards the needs of the country, which has meant concentrating on communications satellites, meteorology and remote sensing. At the same time, since the establishment of India's first rocket-launching facility in 1963 at Thumba almost on the magnetic equator, there has been a substantial programme of ionospheric and magnetospheric studies.

In a country as vast as this, and with a population that is still largely illiterate or semiliterate, the need for a communications satellite has been a live issue since the mid-1960s. An experimental year's work (1975-76) using the US ATS-6 satellite on the Satellite Instruction Television Experiment (SITE), in which 2,400 villages in 6 Indian states received specially made instructional television programmes in agriculture, animal husbandry, health and family planning, is said to have confirmed the value of the concept and to have provided valuable experience in the installation, operation and maintenance of direct reception sets in far-flung areas.

In 1977-79, the Satellite Telecommunications Experiments Project (STEP), using the Franco-German satellite *Symphonie*, permitted the field testing of indigenous communications equipment. SITE and STEP together were the basis for APPLE, a three-axis geosynchronous communications satellite with a C-band transponder, produced by the Bangalore satellite centre of the Indian Space Research Organization (ISRO).

APPLE (Ariane Passenger Payload Experiment) was launched by a European Space Agency Ariane rocket on 19 June 1981 and placed in a geostationary orbit with the aid of an indigenously-produced apogee-boost motor. Although the allotted orbital position (102°E) was somewhat eccentric for Indian coverage, a programme of communications experiments

was carried out successfully, covering computer communications, time division multiple access, spread spectrum techniques and the use of small terminals. This made it possible to proceed to the next step,



The SLV-3 launcher, 18 July 1980.

the development of the Indian National Satellite Communications System INSAT (which in spite of its name is not totally indigenous but used US launch facilities and was built by the Ford Aerospace Corporation in the United States).

INSAT was planned as a unique multipurpose operational space system, providing telecommunications, meteorological and television services from a common satellite. In fact, plans called for the use of two satellites, one acting as back-up. INSAT-1A (launched on 10 April 1982) ran

into deployment difficulties (the solar sail would not open, using up too much fuel and limiting the simultaneous channel operation; then, in September 1982, Earth-lock was lost). Even so, investigation showed the basic design was sound and its successor, INSAT-1B, was launched and deployed in September 1983 without difficulty. ISRO now plans a fully-Indian communications satellite system.

The remote sensing programme has grown naturally out of aerial surveys which began, in India, in 1920. The National Remote Sensing Agency (NRSA), established in 1975, covers both aircraft and satellite observations. Although the NRSA data-processing station at Hyderabad has access to real-time Landsat data, India has developed its own remote-sensing satellites, Bhaskara-1 and 2 (which were launched from the Soviet Union) and Rohini, launched by the indigenous satellite launch vehicle SLV-3.

The plan now is for an indigenous remote-sensing satellite (IRS) which will be a three-axis body stabilized polar Sun-synchronous device carrying an array of solid-state cameras operating in the visible and near-infrared regions and due to be launched in 1986. Data from the remote sensing programme is used for the now-standard applications of geological prospecting, monitoring of crop diseases, ground-water studies, environmental monitoring, meteorology and marine resources, coastal erosion and the mapping of silt outfalls from major rivers.

The first ten years of the programme were considered as experimental. The outcome was summarized last year at a high-level seminar of the user agencies, mainly government ministries, as a stepping-stone towards what has been described as a fully integrated remote-sensing data-base for the country.

Vera Rich

How to run a laboratory

THE intellectual springboard for the space programme is the Physical Research Laboratory at Ahmedabad, founded in 1947 by the late Dr Vikram Sarabhai.

In spite of its connections, the laboratory's programme is interesting because of its diversity. But it is a high-technology laboratory in that it makes a point of using the latest techniques to accomplish even familiar goals — in archaeology and palaeoclimatology, for example.

The palaeoclimate is that of the Kashmir Valley during the Pleistocene. This valley-floor is peppered with lakes and relict lakes from whose sediments it seems possible, by pollen analysis, to reconstruct a record of the climate over the past five million years. Stable isotopes and palaeomagnetism have been measured in cores from 500 or so sites, providing what is thought to be a unique collection of data.

But the Physical Research Laboratory

also houses India's most substantial venture into thermonuclear fusion, partly supported by the US National Science Foundation but with a spanking new torus built with Rs 12 million from the Department of Science and Technology. And the laboratory is building a 1.2 m infra-red telescope to a design developed by Professor P. V. Kulkarni.

The Physical Research Laboratory is full of zip. People are doing things and not just thinking that they might. And one thing leads to another, whence the meteorology, astronomy, tropospheric research and the theoretical programmes to go with them.

The secret of success is to keep small (200 people) and to be grateful that there is no room for expansion. Then, if something gets too big, you set up another laboratory elsewhere. This has already happened for the sounding rocket programme. The Kashmir Valley project may follow soon.

Observation and things observed

A persuasive account has at last been given of the probability spread of quantities that cannot simultaneously be measured: measuring devices enter equally with the systems measured.

EVERYBODY knows that it is not possible simultaneously to measure accurately the position and the momentum of a particle. So much has gone without saying for the past half-century, and the same applies to the members of any other pair of canonically conjugate dynamical variables, say energy and time. More generally, the minimum uncertainties of two simultaneous measurements of, say, position and momentum are inversely related to each other in the sense that the product of the two uncertainties is not less than some constant which turns out to be Planck's constant divided by 2π , called $\hbar$ for short. This is how Heisenberg's Uncertainty principle has instructed us since 1925.

Nothing in what follows is to be read as a contradiction of Heisenberg's principle but only as a gloss on it prompted by an intriguing discussion of the problem of simultaneous measurement by K. Wodkiewicz of the University of Rochester (*Phys. Rev. Lett.* **52**, 1064; 1984). One way of putting his question is to ask what can be said about the statistical distribution of the momentum of some particle, say an electron, whose motion is sufficiently well understood for it to be represented by a wave function which is a solution of Schrödinger's equation. The standard solution for the simplest case of all, a particle moving freely through a force-free region, is elementary — the wave function is a plane wave, and the implication is that the particle is equally likely to be found anywhere in the infinite accessible region. The momentum distribution is equally simple — it consists simply of the momentum corresponding numerically to the wave vector of the plane wave — a single point in momentum space.

More complicated dynamical systems naturally lead to a more complicated dual relationship between the probability distribution of a position coordinate and its conjugate momentum. But if a wave function (in terms of the position coordinate) can be found for the stationary state of the system, it is always possible to calculate the expected value of the momentum, and the successive moments about this mean, by the simple rules of quantum mechanics or wave mechanics. But why not go the whole hog, and from the outset calculate the probability distribution in some two-dimensional space (phase space) whose dimensions represent position (q) and momentum (p)?

Wodkiewicz points out that this

question was first tackled in 1932 by Eugene Wigner, but a footnote to that paper (*Phys. Rev.* **40**, 749; 1932) remarks cryptically that Wigner's solution of the problem "was found by L. Szilard and the present author some years ago for another purpose". Wigner's limited objective in 1932 was to find some way of handling functions representing dynamical systems in which each of a pair of conjugate variables appears, in particular the expression for the total energy of the system consisting of the sum of its kinetic energy (a function of p) and potential energy (a function of q). Wigner's goal was to correct classical Boltzmann statistics for the effects of quantum mechanics that become apparent at low temperatures. The byproduct of his work was a function of both q and p , called $W(p, q)$, with most of the properties that would be expected of a probability distribution in phase space.

Wigner's 1932 result defines the combined distribution of a pair of conjugate variables in terms of the wave function with respect to only one of them, and seems to have stood the test of time in spite of its deficiencies. If, for example $f(q)$ is a wave function for a particle moving in one direction, and thus a solution of Schrödinger's equation, the probability distribution of q if given by $f^*(q)f(q)$ (where the asterisk denotes the complex conjugate and where it is assumed that the wave function has been multiplied by some number that makes the total probability unity). Wigner's dual function $W(p, q)$ is $(\pi\hbar)^{-1} \int f^*(q+t) f(q-t) \exp(2ipt/\hbar) \cdot dt$ and was shown to have many of the properties required of a joint probability distribution. Integrating over one variable would, for example, give the probability distribution for the other.

The only other drawback, which Wigner pointed out, is that the function W can sometimes be negative, and so cannot be a true probability. Even so, nearly half a century later Wigner (with R. F. O'Connell) argued that W is the only function that will satisfy what reasonable people would require of a joint distribution (*Phys. Lett.* **83A**, 145; 1981).

Not so, says Wodkiewicz, who takes an admirably positivist point of view. There is no point in talking about phase-space until procedures have been specified for measuring its coordinates, position q and momentum p . He suggests how the job can be done using a hypothetical pulsed laser whose radiation can interact with a particle

by means of its electrical field.

The consequence of the interaction is that a detected particle is scattered so that the form of its wave function is such that, at a great distance from the point of interaction, information can be gleaned both about position and momentum, but only as allowed by the uncertainty principle. This is the basis of what Wodkiewicz calls his operational definition of a phase-space probability distribution.

Everybody, but not least Professor Eugene Wigner, will be anxious to know how the result resembles the old $W(p, q)$. Put briefly, there are on this occasion two wave functions to be taken account of, one describing the state of the laser beam with which the detected particle interacts (f , say) and the other describing the state of the particle after the interaction (here called g), each of which can be used separately to define a Wigner-like phase-space distribution which may be called $W_f(p, q)$ and $W_g(p, q)$ respectively.

Wodkiewicz's result is geometrically very simple (for one space coordinate). The value of the probability distribution in phase-space at the point (p, q) is obtained by displacing the origin of one Wigner function (a pattern in two dimensions) to that point, multiplying by the other undisplaced Wigner function and then integrating over both variables. In simple language, the result is the overlap between two Wigner functions, one for the measuring system and one for the particle. Conveniently, the result cannot be negative.

This outcome is satisfactory for several reasons, but not least because it puts the observing system and the thing observed on an equal footing. This is precisely what the Copenhagen school would have asked for. Second, as Wodkiewicz points out, the calculation of phase-space probability should survive even if the Schrödinger equation were non-linear (which is one way in which gravitational forces might be taken account of). It goes without saying that Wigner's result can be used more confidently now that the reasons why it is incomplete can be understood. But with all that said, is it not remarkable that after half a century of careful examination of the meaning of quantum mechanics, during which the notion that the measuring instrument must be counted part of the system measured has been repeatedly raised, it is only now that the deficiencies of Wigner's calculation should have been explained?

John Maddox

Carbon cycle

Carbon dioxide circulation through ocean and atmosphere

from Wallace S. Broecker

JUST over three years ago measurements of the CO₂ content of air from polar ice cores were independently published by groups in Bern¹ and Grenoble². Both groups concluded that the CO₂ content of the atmosphere during the last glacial period was about two-thirds of that in post-glacial times. Oceanic and atmospheric contributions to the carbon cycle must have been responsible for changes on such a time scale and two papers on pages 621 and 624 of this issue of *Nature* describe the first results of computer models designed to clarify the roles of the ocean-atmosphere system^{3,4}.

The dramatic changes in atmospheric CO₂ content revealed by the ice cores immediately captured the interest of the geochemical and climatological communities for it was the first clear evidence for significant natural changes in the atmosphere's trace gas content (and hence in its greenhouse capacity). I jumped on the result and tried to explain it⁵.

It was easy to show that it must have originated in the ocean and was not the result of the warming of the ocean surface which accompanied deglaciation. I came up with two viable hypotheses, consistent with the rather strong constraints imposed by the ¹³C record for the surface and deep waters of the sea as recorded in the shells of planktonic and benthic foraminifera in deep-sea sediments.

Both hypotheses involved changes in the relative abundances of carbon to the limiting nutrients, phosphorus and nitrogen. In the first hypothesis, this change was caused by the removal of organic matter to the sediments deposited along the margins of the sea as they were flooded by the water released from the melting glaciers. In the second, the change was induced by a decrease at the onset of post-glacial time in the ratio of carbon to nitrogen and phosphorus in the organic matter falling from the sea surface (see ref. 6 for the detailed arguments).

Early last year the Bern group dropped another 'bomb' on the climate community. Working with samples from a new ice core from the Dye 3 site in Greenland the group showed that the changes in atmospheric CO₂ associated with the close of glacial time occurred in a period of less than 1,000 years; moreover, for several time spans of about 1,000 years within the glacial period, coinciding with warming recorded by ¹⁸O to ¹⁶O shifts⁷, the CO₂ content returned more than half way to its post-glacial value⁸. Since the bulk chemistry of the ocean-atmosphere system cannot change within 1,000-year periods, my sedimentation hypothesis dropped from contention to be

replaced by an alternative hypothesis generated with Taro Takahashi.

We suggest that because carbon dioxide can be transported through the atmosphere from one region of the ocean surface to another while nitrate and phosphate cannot, it is conceivable that changes in atmospheric CO₂ content are induced by changes in the deep-sea ventilation rate⁹, in which case the rise in CO₂ content of the air at the close of glacial time might simply have been the result of a decrease in that rate.

Groups from Princeton, Bern and Harvard have also investigated lower-than-present concentrations of nitrate and phosphate in polar surface water during glacial time. Papers from the first two groups appear on pages 621 and 624 of this issue; the third group has a paper in press in *Journal of Geophysical Research*.

While differing in detail, all three papers call on a more efficient utilization by plants of the nitrate and phosphate reaching the sea surface in polar regions than elsewhere. The resulting drop in the nutrient content of Antarctic surface waters leads to a reduction in the total oxidized carbon (ΣCO_2) content of these waters and also in the CO₂ content of the atmosphere. Knox and McElroy suggest that the more efficient utilization of nutrients is related to changes in the amount of light reaching the polar regions¹⁰; Siegenthaler and Wenk opt to explain it by an increase in the residence time of water at the Antarctic surface⁴; and Sarmiento and Toggweiler conclude that it must be the result of changes in either illumination or residence time³.

Discussion among the interested parties at a recent meeting* (reported in ref. 11) led to agreement on several points. First, the ideas we now have are preliminary probes into a new and very fascinating realm of science, namely how the flow of carbon, nitrogen and phosphorus through the ocean-atmosphere system responds to perturbations in the conditions of our planet's surface.

Second, the record of the ¹³C/¹²C ratio of the ocean and atmosphere held in the ice cores will be the key to deciding the relative importance of changes in organic residue composition, ventilation rates and polar nutrient composition in the low CO₂ concentrations of glacial times. As the carbon incorporated by plants is depleted in ¹³C relative to ¹²C, changes in the efficiency of utilization of surface-water nutrients will lead to changes in the ¹³C/¹²C ratio in the ΣCO_2 of surface water (and hence in

planktonic foraminifera). At the meeting, H. Oeschger of the Bern group indicated that the vital carbon isotope record for the atmosphere based on measurements on CO₂ extracted from ice cores would soon be available. Meanwhile, N. Shackleton (University of Cambridge) revealed that his unpublished ¹³C/¹²C records for planktonic forams from sediment cores taken in the Atlantic sector of the Antarctic (50–52°S) show that the ¹³C/¹²C ratio during peak glacial time is 0.3–0.5‰ more negative than during peak interglacial time. Such a change is at odds with the Antarctic nutrient models. If, therefore, these records prove to typify Antarctic surface waters there will be another casualty among the hypotheses.

Third, the much lower dissolved oxygen content of glacial deep-sea water demanded by the three nutrient-based models (that is, sediment storage, residue composition and polar surface water composition) does not seem to be in evidence in the sedimentary record.

Fourth, if, as seems likely from results presented by Michel Andree of the Bern group, the application of the accelerator ¹⁴C dating method (see these columns, R. E. M. Hedges and J. A. J. Gowlett *Nature* 308, 403; 1984) to hand-picked planktonic and benthic foraminifera will enable direct estimates to be made of the rate of ventilation of the deep sea during glacial time, the results should provide a valuable focus for the next generation of hypotheses.

Just as the results of CO₂ measurement on ice cores have captured the interest of those concerned with climates of the past, they will surely turn the heads of those concerned with climates to come. Will anthropogenic changes in atmospheric CO₂ ultimately force a change in the mode of operation of the ocean? The four teams who are searching for an explanation of the ice-core CO₂ results agree that there must have been major changes in the past in ocean operation, with some unknown sequence of events causing the balance to tip from one mode of operation to another. Will the warming of the planet that will surely occur during the next hundred or so years tip the balance again? □

1. Berner, W., Stauffer, B. & Oeschger, H. *Nature* 275, 53 (1979).
2. Delmas, R. J., Ascencio, J.-M. & LeGrand, M. *Nature* 284, 155 (1980).
3. Sarmiento, J. L. & Toggweiler, J. R. *Nature* 308, 621 (1984).
4. Siegenthaler, U. & Wenk, Th. *Nature* 308, 624 (1984).
5. Broecker, W. S. *Prog. Oceanogr.* 7, 151 (1982).
6. Broecker, W. S. *Scient. Am.* 249 (3), 146 (1983).
7. Dansgaard, W., Clausen, H. B., Gundestrup, N., Hammer, C. V., Johnsen, S. F., Kristindottir, P. M. & Reek, N. *Science* 218, 1273 (1982).
8. Stauffer, B., Hofer, H., Oeschger, H., Schwander, J. & Siegenthaler, U. *Ann. Glaciol.* 5 (in the press).
9. Broecker, W. S. & Takahashi, T. in *Climate Sensitivity and Climatic Processes* (eds Hansen, J. E. & Takahashi, T.) (Maurice Ewing Ser. Vol. 5, Am. geophys. Un., Washington DC, in the press).
10. Knox, F. & McElroy, M. B. *J. geophys. Res.* (in the press).
11. Campbell, P. *Nature* 307, 688 (1984).

* The Chapman Conference on 'Natural Variations in Carbon Dioxide and the Carbon Cycle', held in Tarpon Springs, Florida, 9–13 January 1984.

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Biophysics

Crystallin measurements in the intact eye lens

from Christine Slingsby

A FINE illustration of the more gentle ways of physicists than of biochemists, who have traditionally ripped cells apart to investigate — with great success — their macromolecular organization, is contained in a recent issue of *Proceedings of the National Academy of Sciences U.S.A.* In it, Sun *et al.* report the use of a light-scattering technique to measure the accumulation of δ -crystallin in the intact eye lens of chickens¹.

By focusing a small beam of laser light onto discrete regions of an intact lens and analysing the correlation time of the scattered light intensity fluctuations, Sun *et al.* were able to observe brownian motions within the lens. Because the dimensions of the lens cell are large compared with the wavelength of light, the observed brownian motions are of cytoplasmic constituents of the cells. The motions depend on both the size of the scattering elements and their interactions with other molecules. The observed correlation function is therefore a composite of all the random walks of cytoplasmic constituents over distances comparable to the light wavelength but it is possible, in principle, to sort out molecular details of individual components if they are homogeneously dispersed in a single phase.

The diffusion coefficient of a macromolecule can be determined from the relaxation time of the scattered light intensity fluctuations, which is related to the correlation length by the Stokes–Einstein–Kawasaki–Ferrel formula. When interactions between macromolecules are negligible the correlation length is equal to the hydrodynamic radius. When the correlation lengths of components in cells deviate moderately from those measured in homogeneous solutions, it is because of that ephemeral property of matter — short-range order. On the other hand, if a solution or gel is near a critical point, so that different phases coexist yet are clearly distinguishable, the interactions become long range, the measured correlation length is very large and the solution becomes turbid^{2,3}. The correlation length of δ -crystallin measured within the lens by Sun *et al.* was only 20 Å longer than the hydrodynamic radius measured from a monodisperse solution, in line with evidence that only short-range order is necessary for the transparency of the lens^{4,5}.

The fewer the components within a cell, the more reliable the method of determining quantitative parameters of individual macromolecules. Embryonic chicken lenses are well suited in this respect because the cytoplasm is composed largely of cyto-

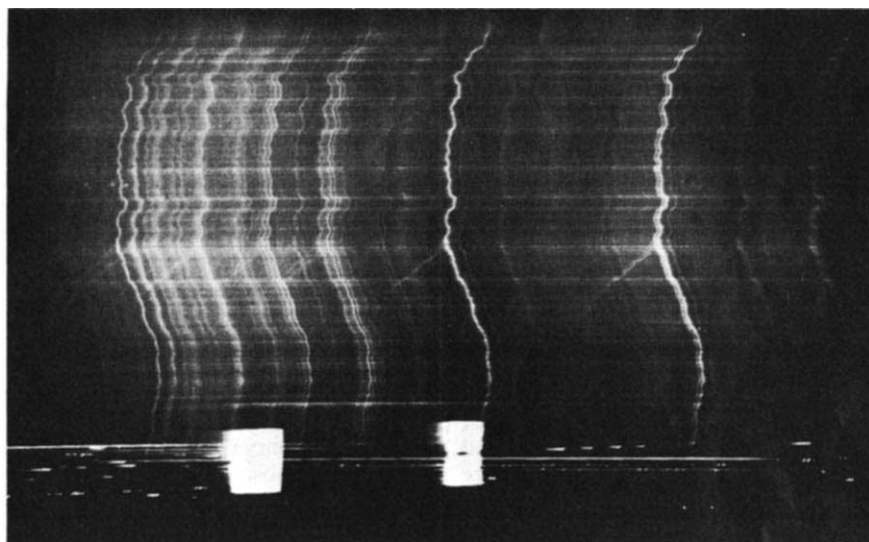
skeletal filaments⁶ and δ -crystallin, a tetramer of molecular weight near 200,000 (ref. 7). In keeping with this description, Sun *et al.* find two correlation lengths, one very large (6,700 Å) and one small (65 Å), corresponding to these components. From the amplitude of the scattered light fluctuations in lenses from embryos and chicks of different ages, Sun *et al.* were able to determine the relative concentration of δ -crystallin within lens cells during the course of the first forty days of development.

Interesting phenomena relating to lens biology discovered by other techniques could now be brought into the field of view of light-scattering science. Lens differen-

tiation requires growth factors from the retina⁷; alterations in the ionic environment of lens cells regulate the size of δ -crystallin subunit to be synthesized⁸; and calcium levels affect the association *in vitro* of δ -crystallin to membranes. These and other physiological responses could now be non-invasively investigated in intact cells. Another obvious target for investigation would be the influence of the cytoskeleton of the lens cell on the mobility of its cytoplasmic constituents. □

1. Sun, S.-T., Tanaka, T., Nishio, I., Peetermans, J., Maizel, J. V. & Piatigorsky, J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 785 (1984).
2. Benedek, G. B. *Polarization, Matter and Radiation* (Presses Universitaires de France, Paris, 1969).
3. Tanaka, T. *Scient. Am.* **244**, 110 (1981).
4. Benedek, G. B. *Appl. Optics* **10**, 459 (1971).
5. Delaye, M. & Tardieu, A. *Nature* **302**, 415 (1983).
6. Maizel, H. *et al. Molecular and Cellular Biology of the Eye Lens* (ed. Bloemendal, H.) 49 (Wiley, New York, 1981).
7. Piatigorsky, J. *Differentiation* **19**, 134 (1981).
8. Shinohara, T. & Piatigorsky, J. *Nature* **270**, 406 (1977).

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ALTHOUGH serious scientific work on the spectra of lightning dates from early this century, with the direct time-resolved photography of Sir Charles Boys and the slitless spectroscopy of E. C. Pickering, the manufacture of diffraction gratings with a large surface area of high accuracy and uniformity produced a revolution in lightning spectroscopy. In 1960, applying a Bausch & Lomb replica transmission grating to an f/8 Aero-Tessar camera, Leon Salanave (then with the Institute of Atmospheric Physics at the University of Arizona) captured this historic stroke of lightning. The spectrum of a single stroke is seen dispersed at 25 Å mm⁻¹ in first order, running from 380 nm at far left to 620 nm at extreme right.

The photograph may be viewed as a three-dimensional graph with position as the ordinate, wavelength as the abscissa and intensity varying from bright knots ('hot spots') to invisibility at any given point. The variation in intensity runs both vertically in space and horizontally in

wavelength. All the brightest lines are attributable to N (II), but other prominent lines belong to N (I), O (I) and O (II). If the spectrum had run on a bit further to the right, the H α line of hydrogen would have been conspicuous; the hydrogen originates in the dissociation of water vapour by the electric spark. Water vapour also makes its presence known explicitly towards the red end of the spectrum, where it is manifest as a broad dark band intersected by lines of N (II).

Writing of the historic significance of the photograph, Salanave comments, "The importance of these slitless spectra, dating from 1960 on, lies in the fact that from them (after time resolution into individual strokes) channel temperatures near peak luminosity could be reliably determined for the first time".

Jon Darius

One of the photographs in an exhibition *Beyond Vision at the Science Museum, London from 19 April. A book with the same title will be published in April by Oxford University Press. Reproduced by courtesy of E. P. Krider, University of Arizona.*

Planetary science

Evolution by bombardment?

from Michael Prather

THE atmospheres of the planets contain a cryptic record of the origin and evolution of our Solar System. The current composition of planetary atmospheres reflects the initial composition of proto-planetary material, the mode of planetary accretion, the release and reabsorption of volatiles by the solid planet, the chemical processes coupling atmosphere and lithosphere, and the escape of atmospheric constituents to the interplanetary medium over the last 4.5×10^9 years. Cameron has now invoked erosion by planetesimal bombardment as an important and hitherto neglected process in the evolution of planetary atmospheres; moreover, he suggests that the formation of the Moon followed a collision between Earth and a planetesimal the size of Mars¹.

While the outer planets (Jupiter and beyond) appear to have retained large quantities of volatiles from the original solar nebula, the inner planets (Mars, Earth and Venus) appear to have no vestige of a primordial atmosphere. Instead, their atmospheres must reflect the substantial processing and differentiation of materials in the early Solar System. But the story is far from simple. The quantities of noble gases in the atmospheres of Mars and Venus are particularly puzzling and have spawned various theoretical models for origins of the terrestrial planets²⁻⁷. A consistent model for the inner planets must account for relative concentrations of 70:1:0.006 for ^{36}Ar on Venus, Earth and Mars respectively and for the smaller range in the relative concentrations (3:1:0.2) of N_2 (the abundance on Mars is corrected for the escape of nitrogen predicted by isotope fractionation). One explanation attributes the abundance of noble gases on Venus to solar wind implantation of the protoplanetary material and associates Mars and Earth with the noble gas component found in many meteorites^{5,6}. This argument is supported by the difference in neon isotope ratios between Venus and Earth, but cannot readily explain the similar ratios of $\text{Ne}/^{36}\text{Ar}$ for all three planets.

Cameron's provocative assertion is that the early atmospheres, which evolved from degassing of the partially molten planet, were significantly eroded by collisions with the remaining interplanetary debris. Accordingly, a planetesimal with dimensions comparable to the scale height of the atmosphere is supposed to have generated a shock wave, ejecting part of the atmosphere above the region of impact. This aspect of the theory is not quantitative and, like most theories of primordial atmospheres, is calibrated after the fact by the present atmosphere. Moreover, Cameron's analogy to calculations of

stellar collisions does not seem appropriate.

A second major premise of Cameron's paper is that the Moon formed from the collision of Earth with a planetesimal the size of Mars. During the suggested impact, siderophile elements such as iron would gravitate to the Earth's core and lithophile elements on the surface would be vaporized. The expanding cloud would condense into large circumterrestrial silicate ring which in turn would gravitationally coalesce into the Moon. In its favour, the mechanism could explain the Moon's lack of both volatiles and a significant iron core. But there are difficulties with the energetics of this ring-Moon system⁸ that Cameron considers but fails to resolve entirely.

Models for early bombardment are regrettably unquantitative and create problems while solving others. One implication of Cameron's theory of lunar creation is the removal of a major portion of the Earth's atmosphere through interaction with the silicate ring. He argues that more than 99 per cent of the original atmospheric content of xenon was lost and that even greater proportions of the lighter noble gases Ar and Kr would have been removed. His theory has to face the fact that the Earth is anomalously low in xenon in comparison with the $^{132}\text{Xe}/^{36}\text{Ar}$ ratio in meteorites. One contested explanation for this anomaly has the 'missing' xenon buried in sedimentary rocks^{9,10}. Cameron's theory requires the opposite, a primordial atmosphere with relatively little

xenon. The only known source for such a mix of noble gases would be the original nebula or, later, the solar wind. That source is generally believed to account for Venus' noble gases, but it would predict unacceptable ratios of $\text{N}/^{36}\text{Ar}$ and $^{20}\text{Ne}/^{22}\text{Ne}$ for the Earth.

Mars' low abundance of ^{40}Ar (derived from decay of ^{40}K with half life of 1.3×10^9 years) is used by Cameron as evidence of an extended period of bombardment and atmospheric erosion. This premise contradicts evidence supporting early loss of Mars' volatiles. The high ratio of $^{40}\text{Ar}/^{36}\text{Ar}$ for Mars (10 times that for Earth) argues for substantial loss of ^{36}Ar before the radiogenic production of ^{40}Ar . A similar interpretation is required by the xenon isotopic composition⁵.

The potential importance of early bombardment in the evolution of the terrestrial atmospheres should not be ignored. Cameron has highlighted some interesting consequences of major collisions in the early Solar System. He has chosen examples which may resolve some of the intriguing puzzles about formation of the terrestrial planets but which fail to explain numerous other constraints imposed by observations. □

1. Cameron, A.G.W. *Icarus* **56**, 195 (1983).
2. Anders, E. & Owen, T. *Science* **198**, 453 (1977).
3. Pollack, J.B. & Black, D.C. *Science* **205**, 56 (1979).
4. Morgan, J.W. & Anders, E. *Geochim. cosmochim. Acta* **30**, 43, 1601 (1979).
5. McElroy, M.B. & Prather, M.J. *Nature* **293**, 535 (1981).
6. Wetherill, G.W. *Icarus* **46**, 70 (1981).
7. Pollack, J.B. & Black, D.C. *Icarus* **51**, 169 (1982).
8. Thompson, A.C. & Stevenson, D.J. *Lunar planet. Sci.* **14**, 787 (1983).
9. Fanale, F.A. & Cannon, W.A. *Earth planet. Sci. Lett.* **11**, 362 (1971).
10. Podosek, F.A., Honda, M.S. & Ozima, M. *Geochim. cosmochim. Acta* **44**, 1875 (1980).

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Cell biology

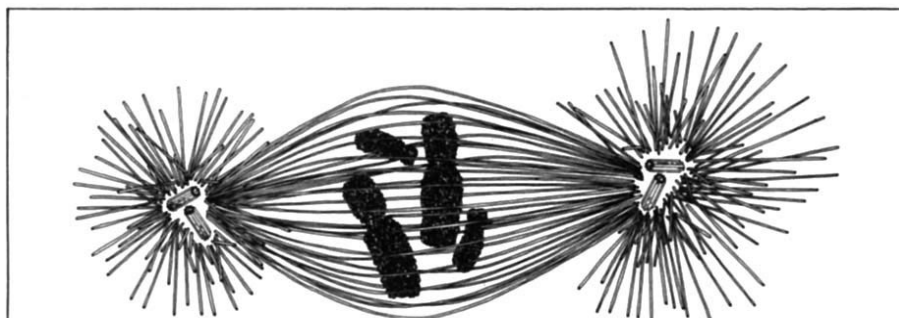
Rheumatoid sera unravel microtubule organizers

from Jeremy S. Hyams

THE complex arrays of microtubules in cultured cells, revealed so spectacularly in recent years by techniques of immunofluorescence, are organized by two types of cellular structure, the centrosome and the kinetochore. Both will nucleate the assembly of microtubules *in vitro*, although it is still debated whether the kinetochore performs that role in the living cell¹. Either way, the composition of both structures and the nature of any changes they might undergo during the cell cycle is of considerable interest; indeed, it is probably not stretching a point to call it the most important current focus of microtubule research. A timely shove to progress in this area has recently come from the unlikely source of the sera of humans suffer-

ing from various rheumatoid conditions. Several publications in the last few months have made use of the autoantibodies in such sera to help characterize centrosomes and kinetochores.

The centrosome is the major microtubule-organizing centre of almost all animal cells and is visible throughout the cell cycle. During interphase, a single centrosome, containing a pair of centrioles, initiates the array of cytoplasmic microtubules; at G_2 , the centrosome divides in two, one for each pole of the mitotic spindle. The kinetochore is only clearly visible at mitotic prophase when one develops on each side of the chromosomal centromere; microtubules from the kinetochore anchor each chromosome to



The mitotic apparatus of a cell in metaphase. Chromosomes, each divided into a pair of chromatids joined only at the centromere, sit in the centre of a spindle composed of microtubules. Some microtubules connect the kinetochores on the centromeres to a centrosome; others connect the two centrosomes, each composed of a pair of centrioles surrounded by amorphous pericentriolar material. From *Cell and Molecular Biology* by E.J. Dupraw (Academic, 1968).

the spindle.

The autoantibodies of most value in the study of kinetochores come from the sera of a subset of rheumatoid patients, those with the so-called CREST (calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, telangiectasia) variant of scleroderma. Such sera, when used as immunofluorescent probes on frozen tissue sections or cultured cell lines, give a characteristic speckled nuclear fluorescence. In mitotic cells, fluorescence is clearly seen to be localized to the centromere region of each chromosome. Immunoelectron microscopy has localized the binding of one CREST serum to the inner and outer plates of the trilaminar kinetochore but not to the underlying centromeric chromatin² and has been used to identify 'prekinetochores' during interphase, the period when they are normally cytologically invisible. Like the centrosome, these small electron-dense spheres duplicate and separate during the G₂ phase of the cell cycle.

Two recent papers have used CREST sera to probe both the molecular composition of the kinetochore and its integration into the chromosome. In one, twelve antisera that stained kinetochores were shown, by immunoblotting, to recognize a 77,000-molecular-weight protein; nine of the sera also recognized an antigen of molecular weight 110,000 (ref. 3). Interestingly, both proteins were present not only in isolated chromosomes but also in chromosome 'scaffolds', the protein framework which persists when metaphase chromosomes are first depleted of histones and then digested with nucleases. In the second paper, other CREST sera were shown to recognize a different spectrum of presumptive kinetochore components⁴. At least four proteins, of 14,000, 20,000, 23,000 and 34,000 molecular weight, are detectable in both interphase and mitotic cells of various cultured lines, suggesting that the structural re-organization of the kinetochore at prophase, which parallels its competence to interact with microtubules, is not accompanied by major compositional changes⁴. The exciting possibility that one or more of these

proteins undergoes some sort of cell-cycle-dependent modification is now experimentally testable.

The anti-kinetochore specificity of CREST sera has also been useful in investigating the role of the kinetochore as a microtubule-organizing centre. Preincubation of lysed mitotic cells with antiserum before incubation with tubulin specifically blocked the assembly of microtubules onto the kinetochore whilst having no effect on growth onto the centrosome⁴. Although there are various interpretations of this finding, the most intriguing is that the two presumptive centres are chemically, and possibly functionally, distinct. An assessment of the extent and significance of the distinction should be helped by those scleroderma patients (a low percentage) who produce autoantibodies not to kinetochores but to centrosomes⁵. One such

serum has now been characterized cytologically⁶. By light and electron immunocytochemistry it stains the cloud of unstructured fuzz that surrounds the two centrioles — the so-called pericentriolar material, which appears to be more involved than the centriole in initiating microtubule assembly. The pericentriolar material remains closely associated with the centrioles, staining as a single bright dot which splits as the cell enters division. In the early divisions of mouse embryo cells, from which centrioles are absent, the pericentriolar material has a different fate, occupying a broad band at the spindle pole during mitosis but becoming undetectable during interphase.

The cellular control of microtubule assembly clearly resides in a limited number of preformed sites, the activity of which is tightly integrated with the cell cycle. The scleroderma sera described here offer the first real molecular probes of these putative microtubule-organizing centres and we shall doubtless be hearing a lot more about them in the next two or three years. □

1. Rieder, C. L. *Int. Rev. Cytol.* **79**, 1 (1982).
2. Brenner, S., Pepper, D., Berns, M. W., Tane E. & Brinkley, B. R. *J. Cell Biol.* **91**, 95 (1981).
3. Earnshaw, W. C., Halligan, N., Cooke, C. & Rothfield, N. *J. Cell Biol.* **98**, 352 (1984).
4. Cox, J. V., Schenk, E. A. & Olmsted, J. B. *Cell* **35**, 331 (1983).
5. Tuffanelli, D. L., McKeon, F., Kleinsmith, D. K., Burham, T. K. & Kirschner, M. *Arch. Derm.* **119**, 560 (1983).
6. Calarco-Gillam, P. D., Siebert, M. C., Hubble, R., Mitchison, T. & Kirschner, M. *Cell* **35**, 621 (1983).

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Astrophysics

Origins of the magnetic fields of neutron stars

from A.G. Lyne

THE origin of the intense magnetic fields of many neutron stars is not clearly understood. Until recently they have commonly been thought to originate from the field of a few gauss of the presupernova stars from which neutron stars evolve; conservation of magnetic flux during the violent collapse of a presupernova star to a neutron star would result in the 10^{12} G surface field strength of the latter that is needed to provide a satisfactory explanation of the spin down, radio emission and polarization of radio pulsars, if they are neutron stars. Now Blandford, Applegate and Hernquist have suggested a quite different origin of the magnetic field of neutron stars by demonstrating, theoretically, two thermally driven instabilities in which a reasonably modest seed field in the neutron star may be amplified to 10^{12} G by the flow of heat from the interior of the star as it cools following supernova collapse¹. Their proposal has a

number of attractions and a number of problems.

Neutron stars are believed to consist of a superfluid superconducting core inside a solid crust, a few kilometres thick, which is not superconducting. Any field present in the core at the time of formation will be trapped there, essentially forever. However, within a kilometre or so of the surface, the electrical conductivity is low enough that ohmic losses could dissipate any embedded magnetic field within a few million years and there is increasing evidence that the magnetization of neutron stars does, indeed, decay².

Assuming that such decay is occurring, Blandford *et al.* argue that the magnetic field of neutron stars must be confined to the surface layers where it can decay. This is just the region where the two instabilities described by Blandford *et al.* will enhance the magnetic field. The evolution of an iso-

lated neutron star is then roughly as follows. At birth the rapidly rotating neutron star has a low magnetic field of maybe 10^8 G, with the interior at a very high temperature. The heat flux from the centre drives the generation of the magnetic field over perhaps 10^4 years and reaches a saturation value of about 10^{12} G. During this time the object is spinning at the rotation rate it had at birth. Once the field has built up, the neutron star will start to spin down as it will be losing rotational energy through electromagnetic dipole radiation at the rotation frequency. Once the heat flow stops, field enhancement will cease and the magnetic field will decay slowly over the next few million years.

Since most pulsars are much older than the 10^4 years suggested as the generation time of their magnetic field, it is not easy to determine whether this mechanism is at play rather than the traditional mechanism in which the field is the 'fossil' field of the presupernova star. But some circumstantial evidence, at least, supports the former explanation.

In particular, observations of the supernova remnant MSH15-52 have recently revealed that it contains a young X-ray and radio pulsar which has a spin-down age of 1,550 years, much less than the estimated 10,000-year age of the remnant³. These apparent ages are clearly inconsistent if the magnetic field was present at birth; but if Blandford *et al.* are right, the neutron star was formed 10,000 years ago and only became a pulsar 1,000 years ago when the magnetic field built up. This delayed 'birth' of pulsar activity may also help to explain the very small number of pulsars observed within supernova remnants: the pulsars appear only after the remnant has lost its identity. However, beaming effects and poor radio sensitivity can more or less explain this anyway — existing techniques only detect one pulsar in every 1,000 that must exist in the Galaxy.

Another interesting argument is based on the measured value of the spin-down braking index for the young pulsar PSR0531+21 in the Crab Nebula. This should take the theoretical value of 3.0, based upon simple electromagnetic theory and a constant magnetic dipole moment⁴. But the observed value of 2.5 is only consistent with this simple theory if the magnetic field is still growing, as expected from the proposal of Blandford *et al.*

Continuing this line of reasoning, one might think that the recently discovered millisecond pulsar PSR1937+21, which has a period of only 1.6 milliseconds and apparently a very weak magnetic field^{5,6} of $\sim 10^8$ G, might be in an early stage with its magnetic field still developing. The absence of any remnant of the supernova explosion suggests, however, that this is not the case and that other explanations⁷⁻¹⁰ are required.

The recently observed dependence of the space velocities of pulsars on their magnetic fields¹¹ may also have a bearing on the

proposal of Blandford *et al.* It is not yet clear how pulsars acquire their high space velocities of a few hundred kilometres per second or how the magnetic field is involved. If the velocity is due to asymmetry in a supernova explosion, then the magnetic field seems to be involved in providing the small amount of asymmetry required and cannot have changed substantially since birth — in which case the mechanism suggested by Blandford *et al.* cannot be at play. Alternatively, the pulsar may be accelerated during its early life by the asymmetric emission of electromagnetic radiation due to an asymmetric magnetic field configuration¹². The absence of any observed relationship between the direction of proper motion and the direction of the rotation axis deduced from linear polarization measurements^{11,13} argues against this process but, if it is nonetheless correct, then the acceleration will occur only after the magnetic field has developed by the field enhancement mechanism of Blandford *et al.*

Blandford *et al.* themselves admit that their theoretical arguments are subject to a number of uncertainties; the observational evidence is also equivocal. It will probably

take the discovery and study of more very young pulsars, like the Crab pulsar and MSH15-52, before it is clear whether they are correct or whether it is still more probable that the magnetic fields of neutron stars are a relic of the fields of their progenitors. □

1. Blandford, R. D., Applegate, J. H. & Hernquist, L. *Mon. Not. R. astr. Soc.* **204**, 1025 (1983).
2. Lyne, A. G., Anderson, B. & Salter, M. J. *Mon. Not. R. astr. Soc.* **201**, 503 (1982).
3. Seward, F. D. & Harnden, F. R. Jr *Asrophys. J. Lett.* **256**, L45 (1982).
4. Manchester, R. N. & Taylor, J. H. *Pulsars* (Freeman, San Francisco, 1977).
5. Ashworth, M., Lyne, A. G. & Smith, F. G. *Nature* **301**, 313 (1983).
6. Backer, D. C., Kulkarni, S. R. & Taylor, J. H. *Nature* **301**, 314 (1983).
7. Alper, M. A., Cheng, A. F., Ruderman, M. A. & Shaham, J. *Nature* **300**, 728 (1982).
8. Fabian, A. C., Pringle, J. E., Ferbut, F. & Wade, R. A. *Nature* **301**, 222 (1983).
9. Aarons, J. *Nature* **302**, 301 (1983).
10. Henrich, H. F. & van den Heuvel, E. P. J. *Nature* **303**, 213 (1983).
11. Anderson, B. & Lyne, A. G. *Nature* **303**, 597 (1983).
12. Harrison, E. R. & Tademaru, E. *Astrophys. J.* **201**, 447 (1975).
13. Morris, D., Radhakrishnan, V. & Shukre, C. S. *Nature* **260**, 124 (1976).

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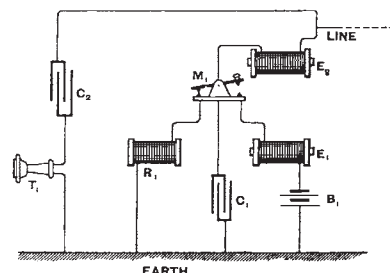
100 years ago

Telephony and telegraphy on the same wires simultaneously.

FOR the last eighteen months a system has been in active operation in Belgium whereby the ordinary telegraph wires are used to convey telephonic communications at the same time that they are being employed in their ordinary work of transmitting telegraphic messages. This system is the invention of M. Van Rysselberghe, whose previous devices for diminishing the evil effects of induction in the telephone service will be remembered.

The method previously adopted by Van Rysselberghe, to prevent induction from taking place between the telegraph wires and those running parallel to them used for telephone work, was briefly as follows:— The system of sending the dots and dashes of code — usually done by depressing and raising a key which suddenly turns on the current and then suddenly turns it off — was modified so that the current should rise gradually and fall gradually in its strength by the introduction of suitable resistances. These were introduced into the circuit at a moment of closing or opening by a simple automatic arrangement worked exactly as before by a key. And as induction from one wire to another depends not on the strength of the current, but on the rate at which the strength changes, this very simple modification had the effect of suppressing induction. Later Van Rysselberghe changes these arrangements for the still simpler device of introducing permanently into the circuit either condensers or else electromagnets having a high coefficient of self induction.

Van Rysselberghe saw that if the telegraphic currents were thus modified and graduated so that they produced no induction in a neighbouring telephone line, they would produce no sound in the telephone if that instrument were itself joined up in the telegraph line. Why this is so will be more readily comprehended if it be remembered that a telephone is sensitive to the changes in the strength of the current if those changes occur with a frequency of some hundreds or in some cases thousands of times *per second*. On the other hand, currents vibrating with such rapidity as this are utterly incompetent to affect the moving parts of telegraphic instruments, which cannot at the most be worked so as to give more than 200 to 800 separate signals *per minute*.



The arrangement is shown where a "graduated" telegraph-set is intercalated into a telephonic system so that both work simultaneously, but independently, through a single line. The combined system at each end of the line will then consist of the telephone-set T_1 , the telegraph instruments (comprising battery B_1 , key M_1 , and Morse receiver R_1), the "graduating" electromagnets E_1 and E_2 , the "graduating" condenser C_1 , and the "separating" condenser C_2 . A single wire between Brussels, Ghent, and Ostend is now regularly employed for transmission by telegraph of the ordinary messages and of the telemeteorographic signals between the two observatories at those places, and by telephone of verbal simultaneous correspondence for one of the Ghent newspapers. From *Nature* 29, 554, 10 April 1884.

Cancer biology

Heritable fragile sites in cancer

from Michelle M. LeBeau and Janet D. Rowley

AMONG the most rapidly advancing areas of cancer research is the study of chromosomal rearrangements in tumours and the determination of their potential role in tumorigenesis. The remarkable concordance between the chromosomal location of human cellular oncogenes (*c-onc*) and the breakpoints involved in certain consistent chromosomal rearrangements associated with various cancers has enhanced the excitement¹. It is now worth examining the extent to which these nonrandom chromosomal rearrangements are associated with the 'fragile sites' of chromosomes.

A fragile site is a region of a chromosome, usually on both chromatids, that fails to become stained. These sites are inherited in a mendelian fashion so that the same locus is involved in any particular family. To date, 17 fragile sites have been identified in the human genome. Of these, 16 are autosomal and 1 is on the X chromosome. Autosomal fragile sites are not associated with phenotypic abnormalities but the X-chromosome fragile site is a marker for one form of X-linked mental retardation²⁻⁴. The expression of 14 of the 17 fragile sites is enhanced by the absence of folic acid in the culture medium. As a result of individual variation and culture conditions, fragile sites are usually seen in less than 50 per cent of lymphocyte metaphase cells. Few data are available on the incidence of individual fragile sites, although the most common fragile site, that at Xq27, is probably present in less than 1 in 1,000 individuals⁴.

The figure summarizes the locations of fragile sites and cellular oncogenes on specific chromosomes, consistent karyotypic abnormalities of the chromosomes and the malignant diseases in which the karyotypic abnormalities are frequently seen. Although sometimes seen in tumours of embryonic origin, in meningiomas and in some other solid tumours, specific chromosomal translocations are a prominent feature in certain types of leukaemia and lymphoma. It is in these diseases that the association of fragile site location and chromosomal abnormalities is most remarkable.

Eleven nonrandom chromosomal abnormalities observed in leukaemia and lymphoma involve six regions known to be fragile sites in some individuals. Thus band p23 of chromosome 6 contains a fragile site and is also the breakpoint in the chromosomal translocation specifically associated with increased bone-marrow basophils in acute nonlymphocytic leukaemia⁵. The translocation t(6;9)(p23;q34) results from an exchange of segments between chromosomes 6 and 9, with breakage occurring at band p23 of the former and

q34 of the latter. The t(8;21)(q22;22) is frequently noted in acute myeloblastic leukaemia with maturation^{6,7} and a fragile site has been identified at 8q22. A fragile site at p21 of chromosome 9 is also the breakpoint in the t(9;11)(p21;q23) most commonly seen in acute monoblastic leukaemia⁸. There are fragile sites at q13 and q23 of chromosome 11, each of which can be the breakpoint in various translocations or deletions in both acute myeloid and lymphoid leukaemias and in lymphoma⁸⁻¹⁰. The fragile site at q22 of chromosome 16 occurs in the band that is involved in both the del(16) and the more frequent inv(16) of acute myelomonocytic leukaemia with abnormal eosinophils^{11,12}. In the case of solid tumours, a fragile site is localized to a region of chromosome 3 that is frequently deleted [del(3)(p14p23)] in small-cell lung carcinoma¹³, and a t(11;22)(q23 or q24;q12) of Ewing's sarcoma^{14,15} involves a breakpoint coincident with the 11q23 fragile site.

As illustrated in the figure, five other chromosomes (2, 7, 12, 17 and 20) which are consistently involved in rearrangements or numerical changes in malignant cells, also contain fragile sites, albeit localized to different chromosomal bands. To summarize, 7 of 17 fragile sites occur at specific chromosomal bands involved in non-

random abnormalities, and 12 of the 13 chromosomes containing fragile sites are involved in nonrandom numerical or structural changes in malignant cells.

If the coincidence of the relationship between chromosomal rearrangements of malignancy and heritable fragile sites is meaningful, one might expect cancer patients with chromosomal rearrangements also to be carriers of fragile sites affecting the involved chromosomes. Preliminary data from three sources suggest that this may be so. Yunis found a correlation between three fragile sites and chromosomal rearrangements in five patients with cancer¹⁶; the (non-malignant) lymphocytes of one of two patients with small-cell lymphoma and a t(11;14)(q13;q32) showed a fragile site at 11q13; a third lymphoma patient who had a t(12;14)(q13;q32) also had a fragile site at 12q13; and two patients with acute myelomonocytic leukaemia and an inversion of chromosome 16 [inv(16)(p13q22)] also carried a fragile site at q22. In a separate study¹⁷ of three patients with the same disease and an inv(16) or del(16)(q22), only one had a fragile site at 16q22. We have observed a fragile site at 16q22 in lymphocytes from four of five patients with acute myelomonocytic leukaemia and an inv(16), but not in five healthy controls (unpublished).

Since recent studies indicate that up to 25 per cent of patients with acute myelomonocytic leukaemia may have an inv(16) in their malignant cells¹², one might predict that families whose members have a fragile

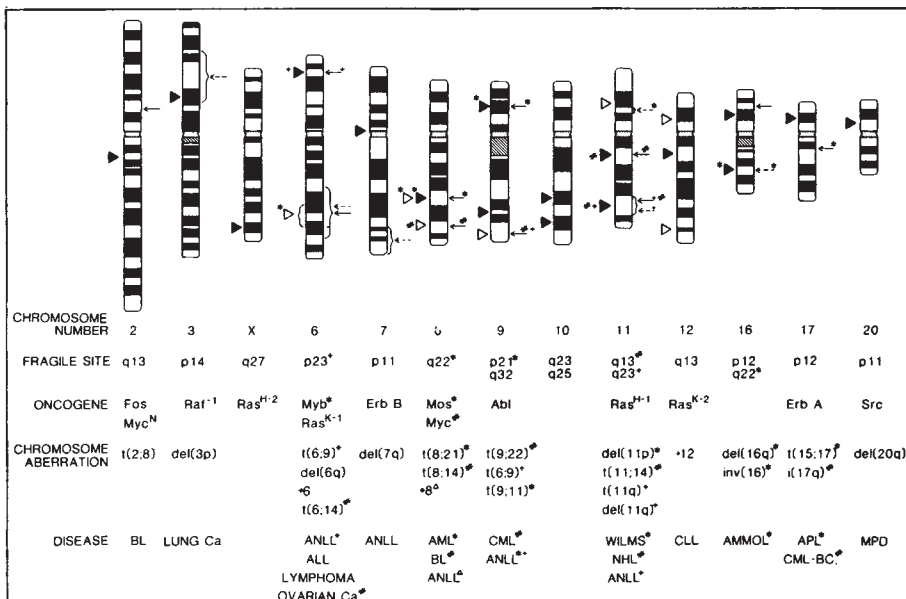


Diagram of chromosomes containing known fragile sites; the chromosome number, fragile site, oncogene, karyotypic aberrations and associated neoplastic diseases are indicated below the chromosome. The arrowheads to the left of each chromosome indicate the bands carrying the fragile site (▶) or cellular oncogene (▷); the arrow(s) to the right of a chromosome identify specific bands involved in consistent translocations (—) or deletions (---) observed in patients having the disorders listed. Additional symbols (*, #, +) link the aberration to a disease. BL, Burkitt's lymphoma; ALL, acute lymphoblastic leukaemia; ANLL, acute nonlymphocytic leukaemia; AML, acute myeloblastic leukaemia; CML, chronic myelogenous leukaemia; AMMOL, acute myelomonocytic leukaemia; AMOL, acute monoblastic leukaemia; BC, blast crisis; NHL, non-Hodgkin's lymphoma; CLL, chronic lymphocytic leukaemia; APL, acute promyelocytic leukaemia; MPD, myeloproliferative disorder. p indicates the short arm and q the long arm of the chromosome; t, del and inv indicate translocation, deletion and inversion.

site at 16q22 would develop the disease and have an inv(16) or del(16) in their malignant cells. Such families have not yet been found. Nor is it known whether individuals with haematological malignancies and other rearrangements at known fragile sites are carriers of a heritable fragile site on the altered chromosome. With respect to this possibility, the t(8;21)(q22;q22) is particularly intriguing because both a fragile site and a cellular oncogene (*c-mos*, the cellular counterpart of the oncogene of Moloney murine sarcoma virus) have been localized to 8q22 (ref. 18). Ten of the twelve autosomes in the figure carry both an oncogene and a fragile site but only in the case of chromosome 8 are they coincident; the cellular oncogenes of chromosomes 6, 8, 9 and 11 are in the same regions as the disease-related breakpoints, with *c-myc* on 6q15-q24, *c-mos* on 8q22, *c-myc* on 8q24, *c-abl* on 9q34 and *c-ras*^{H-1} on 11p15 (ref. 19).

While the structure of fragile sites is unknown, they presumably represent chromosomal segments which do not undergo normal compaction during mitosis. Whether this would serve to predispose the segments to rearrangement is an intriguing, but unanswered, question. More importantly, we are left with the basic questions of whether fragile sites act

as predisposing factors for chromosomal rearrangements in human neoplasia and whether they carry genes whose functions are related to malignant transformation. In either case, the role of selection cannot be overlooked, because a particular rearrangement will result in clonal expansion only if it provides the affected cell with a growth advantage over normal cells. □

1. Rowley, J.D. *Nature* **301**, 290 (1983).
2. Sutherland, G.R. *Am. J. hum. Genet.* **31**, 125 (1979).
3. Sutherland, G.R. *Am. J. hum. Genet.* **31**, 136 (1979).
4. Sutherland, G.R. *Int. Rev. Cytol.* **81**, 107 (1983).
5. Pearson, M.G. *et al. Am. Soc. Hemat.* **62**, 180a (1983).
6. Rowley, J.D. *Ann. Genet.* **16**, 109 (1973).
7. First int. Workshop on Chromosomes in Leukaemia *Br. J. Haemat.* **39**, 311 (1978).
8. Hagemeijer, A. *et al. Cancer Genet. Cytogenet.* **5**, 95 (1982).
9. Rowley, J.D. & Fukuhara, S. *Semin. Oncol.* **7**, 255 (1980).
10. Third int. Workshop on Chromosomes in Leukemia *Cancer Genet. Cytogenet.* **4**, 95 (1981).
11. Arthur, D.C. & Bloomfield, C.D. *Blood* **61**, 994 (1983).
12. LeBeau, M.M. *et al. New Engl. J. Med.* **309**, 630 (1983).
13. Whang-Peng, J. *et al. Science* **215**, 181 (1981).
14. Aurias, A. *et al. New Engl. J. Med.* **309**, 496 (1983).
15. Turc-Carel, C. *et al. New Engl. J. Med.* **309**, 497 (1983).
16. Yunis, J.J. *Science* **221**, 227 (1983).
17. Arthur, D.C. & Bloomfield, C.D. *Am. Soc. Hemat.* **62**, 165a (1983).
18. Neel, B.G., Jhanwar, S.C., Chaganti, R.S.K. & Haywood, W.S. *Proc. natn. Acad. Sci. U.S.A.* **79**, 7842 (1982).
19. Seventh int. Workshop on Human Gene Mapping *Cytogenet. Cell Genet.* **37**, 1 (1984).

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Oceanography

Sea-bed uplift by phase transition

from Peter J. Smith

WHY should the depth of the ocean floor be proportional to the square root of the age (*t*) of the lithosphere in regions of seafloor spreading only for lithosphere of up to about 70 Myr old? Beyond that, in most spreading zones, the ocean floor surface rises higher than the level predicted by the formula, so that for lithosphere 160 Myr old the discrepancy is on average about 800 m (in about 6,000 m).

A number of geophysical models have been proposed to account for this uplift, although only that of Crough (*Tectonophysics* **61**, 321; 1979), later developed by Heestand and Crough (*J. geophys. Res.* **86**, 6107; 1981), has hard data in its favour. One defect of all current geophysical models is that they take the oceanic lithosphere to be physically and chemically homogeneous, whereas, in other contexts, it is widely admitted to be anything but homogeneous. Thus most models assume a predominantly basaltic crust overlying a dry peridotite-rich upper mantle which may or may not include basaltic layers. It is likely that both the basaltic and peridotitic components of the lithosphere will undergo mineralogical and hence physical (especially density) changes as they cool. There seems to be a good case, therefore, for examining what effects such changes might have on ocean floor elevation in the context of the discrepancy between

observation and the $t^{1/2}$ formula. Which is precisely what Wood and Yuen have now done with some success (*Earth planet. Sci. Lett.* **66**, 303; 1983).

Given the uncertainties in lithospheric composition, the possibility of discontinuities in composition and the number of possible changes in mineralogy/petrology in both space and time, this is no easy task. Wood and Yuen therefore chose to concentrate on the phase transition from intermediate-pressure spinel lherzolite to high-pressure garnet lherzolite, a transition widely used to model lithospheric-upper mantle petrology. The spinel region is widely supposed to be the source of mid-oceanic ridge basalts and overlies the garnet region which is regarded as being close to undepleted mantle.

Because the spinel-garnet transition plays a crucial part in many oceanic lithospheric models, it has been extensively studied in the laboratory at high temperatures and pressures. In particular, there are now several sets of data, each showing that as temperature increases, pressure must also increase to allow the transition to take place. In short, the pressure-temperature boundary curve between the spinel and garnet fields has a positive gradient or Clapeyron slope. This is not very encouraging in the context of older cooler lithosphere, for, as Ito has

shown (*Earth planet. Sci. Lett.* **21**, 169; 1974), if the spinel-garnet transition occurs under circumstances in which the Clapeyron slope is positive, the effect will be to depress the ocean floor rather than elevate it. But, because the relationships between pressure and temperature have always been determined at temperatures above 850°C in order to work at practicable reaction rates, it has remained a possibility that the Clapeyron slope is negative at temperatures more appropriate to parts of the oceanic lithosphere.

To investigate that possibility, Wood and Yuen used a combined theoretical-experimental technique developed by (the same) Wood and Holloway (*J. geophys. Res.* **87**, A19; 1982) which essentially involves collection of complete thermodynamic data for each phase involved in the reaction and calculation of the stable mineral assemblage at each required temperature and pressure so that an equilibrium boundary can be pinned down. Wood and Yuen show that in the case of the spinel-garnet transition below about 850°C, the Clapeyron slope is indeed negative.

Armed with the Clapeyron slope, the position of the spinel-garnet transition in the oceanic lithosphere can then be specified. Because, in the relevant temperature region, the Clapeyron slope is negative (that is, as the lithosphere cools and the temperature decreases, the pressure required to effect the spinel-garnet transition needs to be increased), the transition gets deeper with age in the period of interest (>70 Myr). Assuming isostatic compensation and conservation of mass in the lithosphere, it then becomes possible to determine the effect on topography.

It is easy to see what happens qualitatively. As the transition boundary drops with age, the thickness of the less dense spinel phase above the boundary will increase, and hence the level of the ocean floor will lie higher than it would in the absence of the phase transition. Numerically, however, there is no unique solution. The precise effect of the spinel-garnet transition on elevation will depend on the exact composition of the lithosphere, on thermal diffusivity and on the Al_2O_3 content of the lherzolite. But with reasonable ranges for all of these, Wood and Yuen conclude that the spinel-garnet transition could account for 50–300 m of the uplift relative to the $t^{1/2}$ curve at 160 Myr.

Although this is a significant proportion of the observed uplift, it is by no means the whole of it. But then Wood and Yuen are not ruling out contributions either from other phase transitions or from geothermal phenomena. What they have demonstrated is the important principle that the explanation of the elevation of older oceanic lithosphere can no longer be regarded as lying solely within the province of geodynamics.

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A new Earth climate model

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A three-component zonal model of the Earth's climate based on thermohydrodynamic equations for the atmosphere, ocean and terrestrial ice covers is proposed. Sensitivity of the system to changes in initial conditions and also to variations of the solar heat inputs and continent fraction in investigated. Steady-state climate appears to persist in a wide range of perturbed solar constant regimes.

CLIMATE is defined as the statistical ensemble of states of active thermodynamic components of which the most important are the atmosphere, ocean and continental ice. The most reliable tool for climate modelling is simulation of the weather ensemble using three-dimensional hydrodynamic models. When applied to a complete three-component climate model, however, this approach is unacceptable due to the large differences in the inertia of the atmosphere, ocean and continental ice, which are in a ratio of $\sim 1:10^3:10^4$. Simulation of long-period climatic changes, therefore, should be based on simplified models allowing for the evolution and interaction of all three climate components. Available approaches consistent with the above requirements can be divided into three general groups. The first group comprises calculations of the evolution of ice sheets for assigned mass influxes on the surface of glaciers¹⁻⁴. The second group includes two-component atmosphere-continental ice models⁵⁻⁹ in which the climate-forming action of the ocean is allowed for by using either the effective coefficient of horizontal heat diffusion or the inertial seasonal actor in the form of local heat reservoirs. Finally, the well-known investigation of oscillations in a three-component climate system¹⁰ is based on a zero-dimensional model.

This article describes a version of a three-component system combining previously developed one-dimensional models for separate components¹¹⁻¹⁵.

The atmospheric model

The atmospheric model is based on the equations of heat and moisture transport averaged over the latitude circle and thickness of the atmosphere.

$$\frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} (\overline{V\Theta} \cos \varphi) = \frac{g}{p_s C_p} \left(\frac{p_s}{p} \right)^{\kappa} \sum_{i=1}^4 \sum_{K=1}^2 \Lambda_K H_{iK} \quad (1)$$

$$\frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} (\overline{Vq} \cos \varphi) = \frac{g}{p_s} (E - P) \quad (2)$$

Here, H_{2K} , H_{1K} are long-wave and short-wave heat radiation inputs, H_{3K} the turbulent heat input, H_{4K} the heat input due

to evaporation and condensation, Λ_1 and Λ_2 the continent and ocean fractions ($\Lambda_1 + \Lambda_2 = 1$), g the accelerator due to gravity, P the rate of precipitation, a the Earth's radius, φ the latitude, V the meridional component of the velocity, Θ the potential temperature, q the specific humidity, $p = 50,000$ Pa, $p_s = 100,000$ Pa, C_p the specific heat of air at constant pressure, $\kappa = R/C_p$, R the air gas constant, E the rate of evaporation. It is assumed that the meridional heat transport is realized by the non-stationary synoptic turbulence parameterized as recommended in ref. 16: $\overline{V\Theta} = -k |\partial\Theta/\partial\varphi| \partial\Theta/\partial\varphi$, $\overline{Vq} = -k |\partial\Theta/\partial\varphi| \partial q/\partial\varphi$, where k is the coefficient of horizontal diffusion. Calculation techniques for the heat inputs are based on the recommendations given in ref. 17. For potential temperature we thus obtain the ordinary second-order differential equation which is solved numerically with boundary conditions $\partial\Theta/\partial\varphi = 0$ at $\varphi = \pm \pi/2$. The vertical profile of specific humidity is assumed to be known^{14,17}. The rate of precipitation, P , is calculated as the residual term of equation (2). The surface temperature of the continents, sea and terrestrial ice covers is calculated using the equation of local heat budget: $\sum_{i=1}^4 G_i = 0$ (here, G_1 , G_2 are short-wave and long-wave heat radiation inputs¹⁷, $G_3 = -H_{31}$, $G_4 = -\mathcal{L}E$ (where $\mathcal{L}$ is the specific heat of evaporation)).

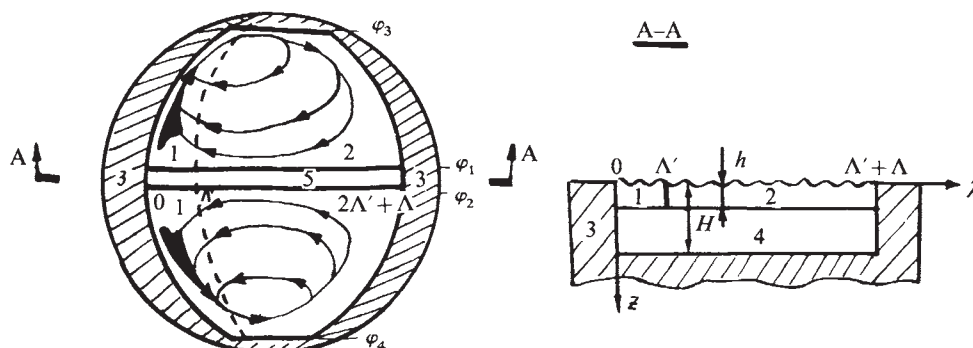
The ocean model

In an ocean of angular width Λ_2 , we separate a layer of the seasonal and main thermocline of depth h and an abyssal layer of thickness $H - h$ (where H is the ocean depth). The oceanic upper layer is divided into an open ocean of width Λ and a western boundary layer of width $\Lambda' \ll \Lambda$ to compensate for the meridional mass transport in the open ocean (Fig. 1).

Reduced to divergent form, the equations of heat conductivity and continuity are averaged over the vertical and along the latitude circle within each region:

$$\frac{\partial}{\partial t} \int_0^{\Lambda'} \int_0^h T dz d\lambda + \frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} \left(\cos \varphi \int_0^{\Lambda'} \int_0^h VT dz d\lambda \right) + \frac{1}{a \cos \varphi} \int_0^h UT dz \Big|_{\Lambda'} = \frac{1}{\rho c} \int_0^{\Lambda'} (Q - Q_1) d\lambda \quad (3)$$

Fig. 1 Ocean model, 1, Western boundary layer; 2, open ocean; 3, land; 4, deep ocean; 5, equatorial belt ($\varphi_2 \leq \varphi \leq \varphi_1$); A-A, vertical section.



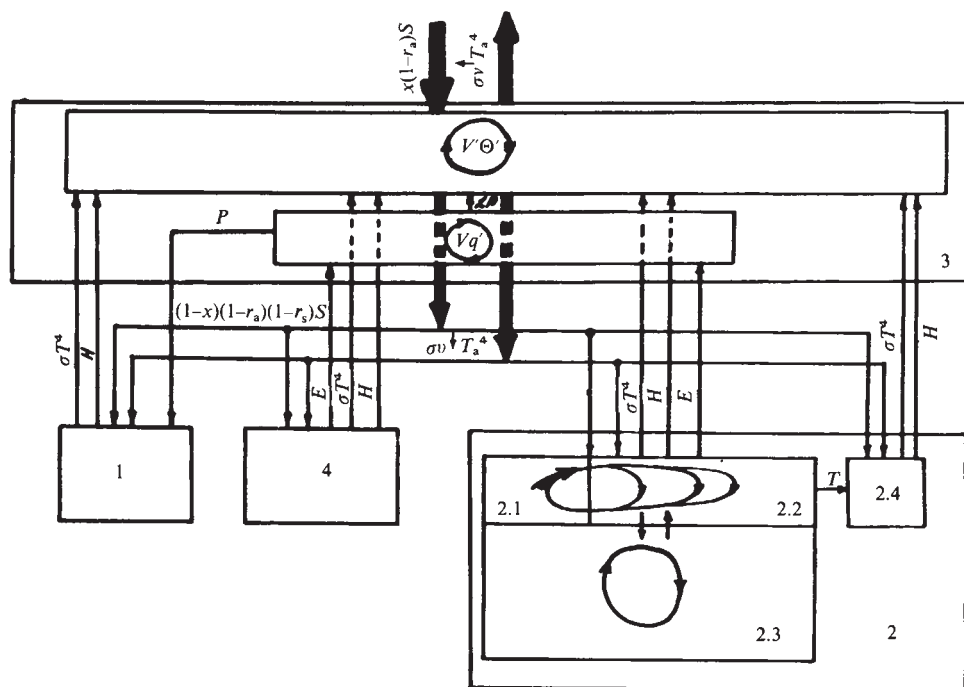


Fig. 2 Schematic representation of climate system. 1, Glaciers (equation (9) and equation of local heat budget); 2, Ocean: 2.1, western boundary layer (equations (3), (4)); 2.2, open ocean (equations (5), (6)); 2.3, deep ocean (equation (7)); 2.4, sea ice (equation of local heat budget). 3, Atmosphere (equations (1), (2)). 4, Ice-free land (equation of local heat budget). S , input of short-wave radiation at the top of the atmosphere; r_a , atmospheric albedo; r_s , underlying surface albedo (different for ice, land and sea); T_a , atmospheric temperature; σT_a^4 , long-wave radiation fluxes; H , E , vertical turbulent fluxes of heat and moisture, P , precipitation; x , v^+ , v^- , atmospheric parameters^{14,17}.

where t is the time, T the temperature, z the depth, λ the longitude, U , V the longitude and meridional components of the velocity, ρ , c the seawater density and specific heat.

$$\frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} \left(\cos \varphi \int_0^{\Lambda'} \int_0^h V dz d\lambda \right) + \frac{1}{a \cos \varphi} \int_0^h U dz \Big|_{\Lambda'} = 0 \quad (4)$$

$$\begin{aligned} & \frac{\partial}{\partial t} \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h T dz d\lambda + \frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} \\ & \times \left(\cos \varphi \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h VT dz d\lambda \right) \\ & - \frac{1}{a \cos \varphi} \int_0^h UT dz \Big|_{\Lambda'} = \frac{1}{\rho c} \int_{\Lambda'}^{\Lambda'+\Lambda} (Q - Q_1) d\lambda \end{aligned} \quad (5)$$

$$\begin{aligned} & \frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} \left(\cos \varphi \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h V dz d\lambda \right) \\ & - \frac{1}{a \cos \varphi} \int_0^h U dz \Big|_{\Lambda'} = 0 \end{aligned} \quad (6)$$

$$\begin{aligned} & \frac{\partial}{\partial t} \int_0^{\Lambda'+\Lambda} \int_h^H T dz d\lambda \\ & + \frac{1}{a \cos \varphi} \frac{\partial}{\partial \varphi} \left(\cos \varphi \int_0^{\Lambda'+\Lambda} \int_h^H VT dz d\lambda \right) \\ & = \frac{1}{\rho c} \int_0^{\Lambda'+\Lambda} Q_1 d\lambda \end{aligned} \quad (7)$$

Here, $Q = \sum_{i=1}^4 G_i$ is the heat exchange of the oceanic upper layer with the atmosphere, and Q_1 the heat exchange between the upper and abyssal layers. The vertical temperature profile in the ocean is assumed to be known

$$T = \{ T_B + (T_s - T_B) \nu(z) \text{ at } z \leq h, T_B \text{ at } z > h \} \quad (8)$$

where $\nu(0) = 1$, $\nu(h) = 0$, T_s is the ocean surface temperature and T_B is the abyssal temperature. The western boundary layer receives latitude-mean heat and mass flows, with the velocity

calculated using the geostrophic relationship: $fu = -1/\rho_0 \cdot \partial p / (\partial \varphi)$; $\partial p / \partial z = \rho g$; $p = 0$ at $z = 0$; $\rho = \rho_0 [1 - \alpha(T - T_0)]$, where f is the Coriolis parameter, ρ_0 the density of seawater and α the coefficient of thermal expansion of the water.

Meridional heat fluxes in the upper layer are written in the form of the product of mean temperature and net flux:

$$\begin{aligned} & \int_0^{\Lambda'} \int_0^h VT dz d\lambda \approx T_1 \int_0^{\Lambda'} \int_0^h V dz d\lambda; \\ & \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h VT dz d\lambda \approx T_2 \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h V dz d\lambda. \end{aligned}$$

In the equatorial belt (region 5 in Fig. 1), where the geostrophic relationship is not valid, meridional advection is not taken into account and the surface temperature is calculated using the equation of the local heat budget. In the abyssal region, the meridional heat transport is described in terms of the diffusion mechanism:

$$\int_0^{\Lambda'+\Lambda} \int_h^H VT dz d\lambda = (\Lambda' + \Lambda) (H - h) K \partial T_B / (\partial \varphi).$$

Heat exchange between the upper and abyssal layers is calculated using the formula $Q_1 = k \rho c (T_s - T_B) / h$. Equations (3) and (5) are ultimately reduced to ordinary first-order equations with respect to T_1 and T_2 . To these is added a second-order equation with respect to T_B .

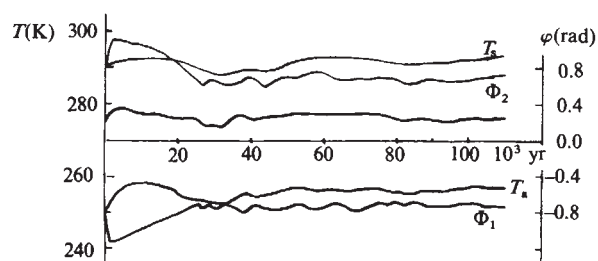


Fig. 3 Example of system evolution. Φ_1 , Φ_2 , Latitudinal extent of southern and northern ice sheets; T_a , T_B , T_s , mean atmospheric, deep ocean and ocean surface temperatures.

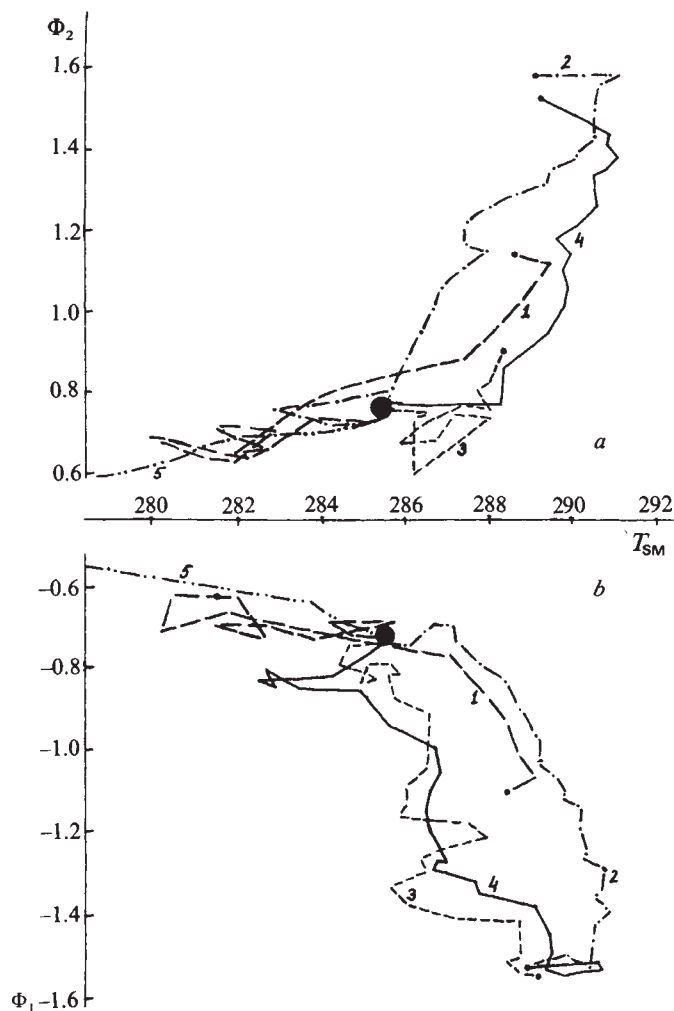


Fig. 4 Climate system evolution for different initial conditions: 1, $\Phi_1 = -\pi/4$; $\Phi_2 = \pi/4$; $T_a = 250$ K; $T_s = 290$ K; $T_B = 275$ K. 2, $\Phi_1 = -\pi/2$; $\Phi_2 = \pi/2$; distributions of T_a , T_s , T_B are present functions. 3, $\Phi_1 = -\pi/2$; $\Phi_2 = \pi/4$; distributions of T_a , T_s , T_B are present functions. 4, $\Phi_1 = -\pi/2$; $\Phi_2 = 0$; distributions of T_a , T_s , T_B are present functions. 5, $\Phi_1 = 0$; $\Phi_2 = 0$; $T_a = 240$ K; $T_B = 275$ K; $T_s = 275$ K. Region of steady-state oscillations is blackened. System position on phase plane is marked every 3,000 yr, T_{SM} , continental temperature. *a*, Phase plane, Φ_2 - T_{SM} ; *b*, phase plane, Φ_1 - T_{SM} .

At

$$\varphi = \varphi_3(\varphi_4) \int_0^{\Lambda'} \int_0^h VT \, dz \, d\lambda + \int_{\Lambda'}^{\Lambda'+\Lambda} \int_0^h VT \, dz \, d\lambda = 0$$

and $\partial T_B / \partial \varphi = 0$. The system as a whole is solved by iterations.

The ice sheet model

The extent of the ice sheet is defined by the integral mass balance:

$$\frac{\partial(h^*S^*)}{\partial t} = a^*S^* \quad (9)$$

where S^* is the glacier area, h^* , the area-mean thickness of the glacier, is obtained using the solution to the ice flow equations written in more¹¹ or less¹² complete form depending on the degree of detail required, and a^* is the difference between accumulation and ablation.

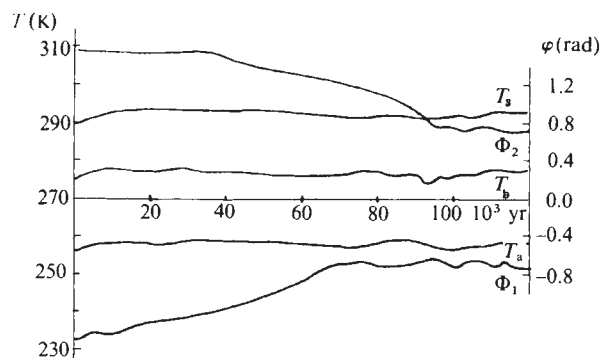


Fig. 5 System evolution for initial conditions of ice-free Earth.

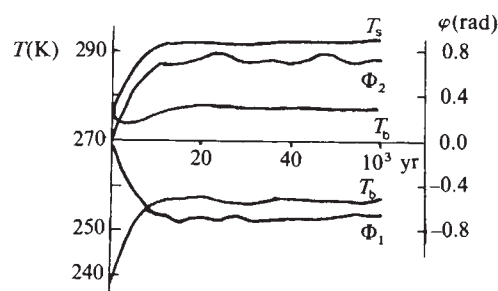


Fig. 6 System evolution for the case when at the initial moment of time all the land was covered by glaciers.

The three-component system

The entire climate system is represented schematically in Fig. 2; heat and mass exchange are indicated by arrows. Short-wave radiations at the top of the atmosphere and the continental fractions are specified for calculating climate evolution. The time-step for ice sheets is taken to be 1,000 yr. Accumulation is determined by precipitation calculated in the atmospheric part and ablation is defined in terms of surface temperature using the empirical relations given in ref. 18. The ocean evolves with a time-step of 100 yr, exchanging heat and moisture with the atmosphere. If at some latitude, the ocean surface temperature reaches freezing point, sea ice is assumed to have been formed there. The non-inertial atmosphere and ice-free continents correspond to oceanic conditions.

Figure 3 shows the evolution for the present flux of solar radiation at the top of the atmosphere and $\Lambda_1 = 0.33 = \text{constant}$, $\varphi_{1,2} = \pm 2.5^\circ$; $\varphi_{3,4} = \pm 87.5^\circ$. Initial conditions are given as the position of ice sheet boundaries assigned at latitudes $\Phi_1 = -\pi/4$, $\Phi_2 = \pi/4$; the glacier thickness is taken to be 2,000 m and the vertical mean atmospheric temperature, ocean surface temperature and deep ocean temperature everywhere are assumed to be: $T_a = 250$ K, $T_s = 290$ K and $T_B = 275$ K. At subsequent times, the glaciers retreat to higher latitudes due to the assigned initial high ocean surface temperature in polar regions. This causes an increase in the mean values of atmospheric and ocean temperature. At the same time, a change occurs in the zonal profile of T_a , T_s , T_B and of continental temperature T_{SM} (not shown in the figure), in accordance with the distribution of the solar heat inputs, which after several thousand years causes the glaciers to spread to lower latitudes.

The characteristic relaxation time of the system is defined by the evolution of the least responsive box, the ice sheets, and is of the order $\tau_1 = h^*/a^* \approx 10^4$ yr. In an ocean with maximum time constant, an abyssal temperature regime is established: $\tau_2 = h(H-h)/k \approx 10^2$ yr, $\tau_2 \ll \tau_1$. The system oscillations become ultimately steady in the vicinity of the point which we shall with certain reservations identify with the present climate. The point is that meridional distributions of the temperature,

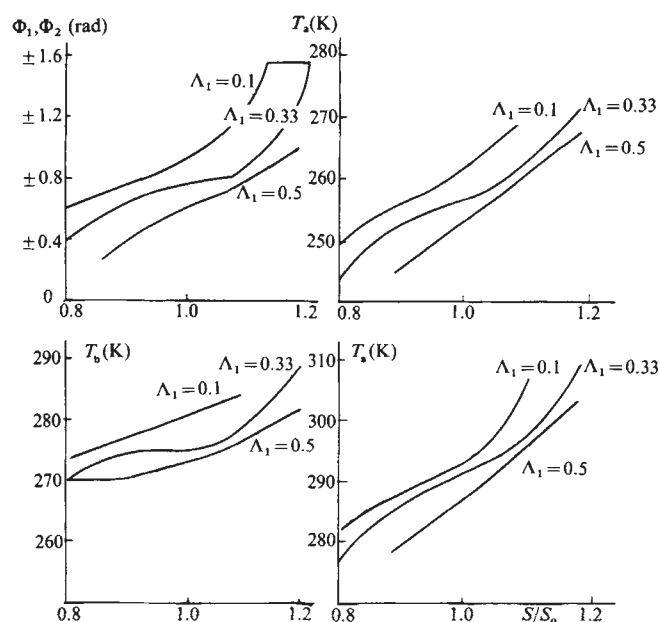


Fig. 7 Sensitivity of climate system to changes in solar radiation flux S/S_0 and continent fraction. Ordinate axes are steady-state values of space-mean quantities.

planetary albedo and heat and moisture flows calculated for this state using the model are in qualitative agreement with empirical data. At the same time, however, there are also differences due to the strongly idealized distribution of land and sea (Fig. 1). For example, the presence of land in the model at latitudes higher than 70° N led to the formation of a large ice sheet there, overcooling of the atmosphere and continents of the northern polar region and spreading of the glacier south of the present limits. The model glacier of the southern polar region spread nearer to the Equator than its present analogue, the Antarctic Ice Sheet bounded by the ocean. In the equatorial region, the model continent fraction is greater than real values, therefore the calculated temperatures at the Equator are somewhat higher than those measured. The mean global values of the quantities are in good agreement with measurements. The atmospheric temperature was 255 K (compare with 256 K in ref. 19), the continental temperature, 286 K (288 K in ref. 20), and the ocean surface temperature and mean temperature of ocean waters appeared to be, respectively 290 and 278 K (291 and 279 K in ref. 21). We note that in ref. 14, where the climate of the atmosphere and continents was calculated at an assigned ocean surface temperature and present fraction of the continents, using the same equations as in the three-component model in question, the meridional temperature profiles were quite close to measured profiles.

Figure 4 shows the sensitivity of the system to changes in the initial conditions. It can be seen that the trajectories on the

phase plane originating at different points converge at the same point, curve 2 corresponding to the initial conditions of an ice-free Earth and curve 5 to glaciers covering all of the land at $t=0$. Evolution of the system for cases 2 and 5 is shown in greater detail in Figs 5 and 6 (curve 1 corresponds to Fig. 3). Note, however, that the initial conditions of version 5 do not quite correspond to the condition of a 'white Earth' because the ocean surface temperature at $t=0$ is assigned a value somewhat higher than freezing point. Furthermore, it is impossible to estimate the stability of the hypothetical state of a 'white Earth', as the heat and momentum fluxes through sea ice are assumed to be zero and the model mechanism of heat transport by oceanic currents no longer corresponds with reality.

Figure 7 shows the sensitivity of the system to changes in the flux of solar radiation and continent fraction. For the present continent fraction, the regime of an ice-free Earth appears to be reached by a 20% increase in the solar constant, while the state of a 'white Earth' is not obtained even if solar radiation is reduced by 20% although the glaciers do reach 23° N and S and the atmospheric and ocean surface temperatures fall to 245 and 275 K respectively. If we use the estimates accepted in the theory of climate, then $S dT_a/dS/100 \approx 0.9$ K and $S d\Phi/dS/100 \approx 1.7^\circ$ of latitude, that is, lower than similar estimates obtained previously^{22,23} using energy balance climate models (for a review of this problem, see ref. 24). This lack of correspondence is due to the different approach used to describe changes in the albedo. In one-component climate models of energy balance (for example, refs 25–27), the albedo is defined by the underlying surface local temperature, whereas in our model, the continental albedo depends on the extent of the ice sheet calculated using equation (9) and the ocean albedo depends on the area of sea ice. Increasing solar radiation leads to rising temperature and increased glacier melting, but also to enhanced global evaporation and, consequently, mass influx to the ice sheet surface. Moisture transport towards polar latitudes is intensified due to the growing difference of the Equator–Pole temperatures. The glaciers retreat (and the albedo decreases), but the retreat is appreciably compensated for by increasing precipitation on their surface. For decreased solar radiation, reverse processes take place. This is indicative of the stability of the Earth's climate in a wide range of perturbed solar constant regimes.

An enhanced continent fraction makes a climate colder: ocean and atmospheric temperature decreases and glaciers spread closer to the Equator and vice versa (see also refs 28, 29).

This article formulates the simplest version of a three-component climate system. Our model attempts to take into account all the main mechanisms of interaction between climatic components. Considerable refinement and further detailed study are possible within the framework of this general approach; for example, generalization of the model ocean for the real distribution of land and sea, and allowance for seasonal variations (mainly, snow and ice covers) will make it possible to obtain more accurate estimates and to come close to mathematical modelling of the Ice Ages.

Received 30 August 1983; accepted 6 January 1984.

- Weertman, J. *Nature* **261**, 17–20 (1976).
- Birchfield, G. E. *J. geophys. Res.* **82**, 4909–4913 (1977).
- Oerlemans, J. *Nature* **287**, 430–432 (1980).
- Pollard, D. *Nature* **296**, 334–338 (1982).
- Pollard, D. *Nature* **272**, 233–235 (1978).
- Kallen, E., Grafoord, C. & Ghil, M. *J. Atmos. Sci.* **36**, 2292–2303 (1979).
- Pollard, D., Ingersoll, A. P. & Lockwood, J. C. *Tellus* **32**, 301–319 (1980).
- Ghil, M. & Le Treut, H. *J. geophys. Res.* **86**, 5262–5270 (1981).
- Birchfield, G. E., Weertman, J. & Lunde, A. T. *J. Atmos. Sci.* **39**, 71–87 (1982).
- Sergin, V. Ya. *J. geophys. Res.* **84**, 3191–3204 (1979).
- Verbitsky, M. Ya. *Dokl. Akad. Nauk SSSR* **256**, 1333–1337 (1981).
- Kvasov, D. D. & Verbitsky, M. Ya. *J. Glaciol.* **28**, 267–272 (1982).
- Verbitsky, M. Ya. & Chalikov, D. V. *Dokl. Akad. Nauk SSSR* **263**, 451–453 (1982).
- Verbitsky, M. Ya. & Chalikov, D. V. *Izv. Akad. Nauk SSSR Ser. Phys. Atmos. Ocean* **18**, 1011–1017 (1982).
- Verbitsky, M. Ya. & Chalikov, D. V. *Dokl. Akad. Nauk SSSR* **269**, 1195–1198 (1983).

- Stone, P. H. & Miller, D. A. *J. Atmos. Sci.* **37**, 1708–1721 (1980).
- Saltzman, B. in *Global Atmospheric Research Program* Vol. 2, 803–841 (1979).
- Khodakov, V. G. *Met. Gidrol.* **7**, 48–51 (1965).
- Oort, A. H. & Rasmusson, E. M. *Atmospheric Circulation Statistics* Vol. 5, 323 (NOAA Prof. Pap., 1971).
- Monin, A. S. & Shishkov, Yu. A. *A History of Climate* (Gidrometeoizdat, Leningrad, 1979).
- Stepanov, V. N. *The World Ocean* (Znanie, Moscow, 1974).
- Golitsin, G. S. & Mokhov, I. I. *Izv. Zhuk. Nauk SSSR, Ser. Phys. Atmos.* **14**, 803–814 (1978).
- Held, I. M., Linder, D. I. & Suarez, M. J. *J. Atmos. Sci.* **38**, 1911–1927 (1981).
- Budyko, M. I. *Climate in the Past and in the Future* (Gidrometeoizdat, Leningrad, 1980).
- North, G. R. *J. Atmos. Sci.* **2033**–2043 (1975).
- Thompson, S. C. & Schneider, S. H. *J. geophys. Res.* **84**, 2401–2414 (1979).
- Bhattacharya, K., Ghil, M. & Vulis, I. L. *J. Atmos. Sci.* **39**, 1747–1773 (1982).
- Barron, E. J., Sloan, J. C. & Harrison, C. G. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **30**, 17–40 (1980).
- Thompson, S. C. & Barron, E. J. *J. Geol.* **89**, 143–167 (1981).

Nucleotide sequence of 3-hydroxy-3-methyl-glutaryl coenzyme A reductase, a glycoprotein of endoplasmic reticulum

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The nucleotide sequence of a 4.8-kilobase mRNA for hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase, the endoplasmic reticulum enzyme that controls cholesterol biosynthesis, shows that it is a protein of 887 amino acids (molecular weight 97,092) which contains three potential sites for asparagine-linked glycosylation. The reductase is a transmembrane glycoprotein, but in contrast to many other transmembrane glycoproteins, it lacks a cleavable or hydrophobic NH₂-terminal signal sequence.

INTENSE current interest in the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase) stems from the observation that transcription of its messenger RNA is susceptible to negative feedback regulation mediated by cholesterol and other isoprenoid products, a regulatory action that controls the cholesterol level in cells and in plasma¹⁻⁴. HMG CoA reductase synthesizes mevalonate which is used for the production of cholesterol and other isoprenoids such as dolichol, ubiquinone and the isopentenyl group of transfer RNAs¹. The reductase is also of considerable interest because it is a glycoprotein that remains inserted in the endoplasmic reticulum (ER) membrane after its synthesis and glycosylation there; much less is known about the primary structure and mechanisms for membrane insertion of ER-associated glycoproteins than of glycoproteins that are exported to other sites after their synthesis and glycosylation in the ER.

Studies of reductase at the molecular level have been made possible by the recent development of a line of cultured Chinese hamster ovary (CHO) cells in which the reductase gene has been duplicated (amplified) 15-fold²⁻⁴. These cells, designated UT-1 cells, were obtained through adaptation of CHO cells to growth in increasing concentrations of compactin, a competitive inhibitor of reductase². In UT-1 cells the levels of reductase mRNA⁴ and the protein itself² are increased more than 100-fold compared with parental CHO cells. The reductase in UT-1 cells is a transmembrane glycoprotein with a molecular weight (M_r) of ~97,000 that contains one or more asparagine-linked high-mannose carbohydrate chains⁵. It is located in hexagonally-packed, smooth tubular membranes designated crystalloid ER^{2,6}.

The high levels of reductase mRNA in UT-1 cells permitted the earlier isolation³ of a recombinant plasmid containing a 1.4-kilobase (kb) cDNA that was complementary to part of the 3'-untranslated region of reductase mRNA. We have now isolated several overlapping cDNAs (spanning 4.8 kb in length) that encompass the entire coding region and much of the 5'- and 3'-flanking regions of reductase mRNA. From the nucleotide sequence of these cDNAs, we have now deduced the primary amino acid sequence of the 97,000- M_r reductase protein which is presented and analysed here.

Nucleotide and amino acid sequences

Five overlapping cDNAs of varying length were isolated, and their nucleotide sequences were determined by a combination

of the methods of Maxam and Gilbert⁷ and Sanger *et al.*⁸, as outlined in Fig. 1 legend. The longest cDNA (pRed-227, 4.5 kb) encompasses the entire coding region as well as 163 base pairs (bp) of 5'-untranslated region and 1,650 bp of 3'-untranslated region. Because of the unusually large size of reductase mRNA and the lack of pre-existing protein sequence data, special care was taken to guard against sequencing errors. In the coding region, both strands of the cDNA were sequenced, usually by both of the sequencing methods. In the 5'- and 3'-noncoding regions, 90% of the sequence was also established for both strands of the DNA. The sequence of the 5'-untranslated region was confirmed by sequencing the corresponding genomic fragment from cloned hamster DNA.

Figure 2 shows the nucleotide sequence of reductase cDNA and the predicted amino acid sequence of the protein. The mRNA encodes a protein of 887 amino acids with a M_r of 97,092, a value that agrees with the most recent estimates of the M_r of reductase by SDS-gel electrophoresis⁵. To confirm the amino acid sequence at the NH₂-terminus, we used radiochemical sequencing. UT-1 cells were cultured in the presence of ³⁵S-methionine or ³H-phenylalanine and the immunoprecipitated reductase was subjected to Edman degradation (Fig. 3). ³⁵S-methionine was found at positions 1, 8, 31, 32, 34 and 36; ³H-phenylalanine was found at positions 6 and 12. These positions corresponded to those predicted by the cDNA sequence (Fig. 2).

To confirm the amino acid sequence at the COOH-terminus, we prepared a synthetic peptide corresponding to the last 14 amino acids of the predicted protein sequence. In experiments to be reported elsewhere, we showed that antibodies raised against this peptide react specifically with the reductase, as determined by immunoprecipitation and immunoblotting techniques. Thus, the predicted amino acid sequence of reductase was correct at both ends.

Signal sequence

A transmembrane orientation for reductase was suggested earlier from two observations: (1) the active site of the enzyme is present on the cytoplasmic side of the membrane, from which it can be released by proteolytic digestion⁵; and (2) the enzyme contains at least one asparagine-linked high-mannose carbohydrate chain that remains associated with the membrane after proteolysis and that is presumably on the luminal side⁵. The mechanism by which reductase is inserted into the ER membrane is unknown.

To date, only one other class of ER proteins, the ribophorins, have been shown to contain carbohydrate⁹. Both members of

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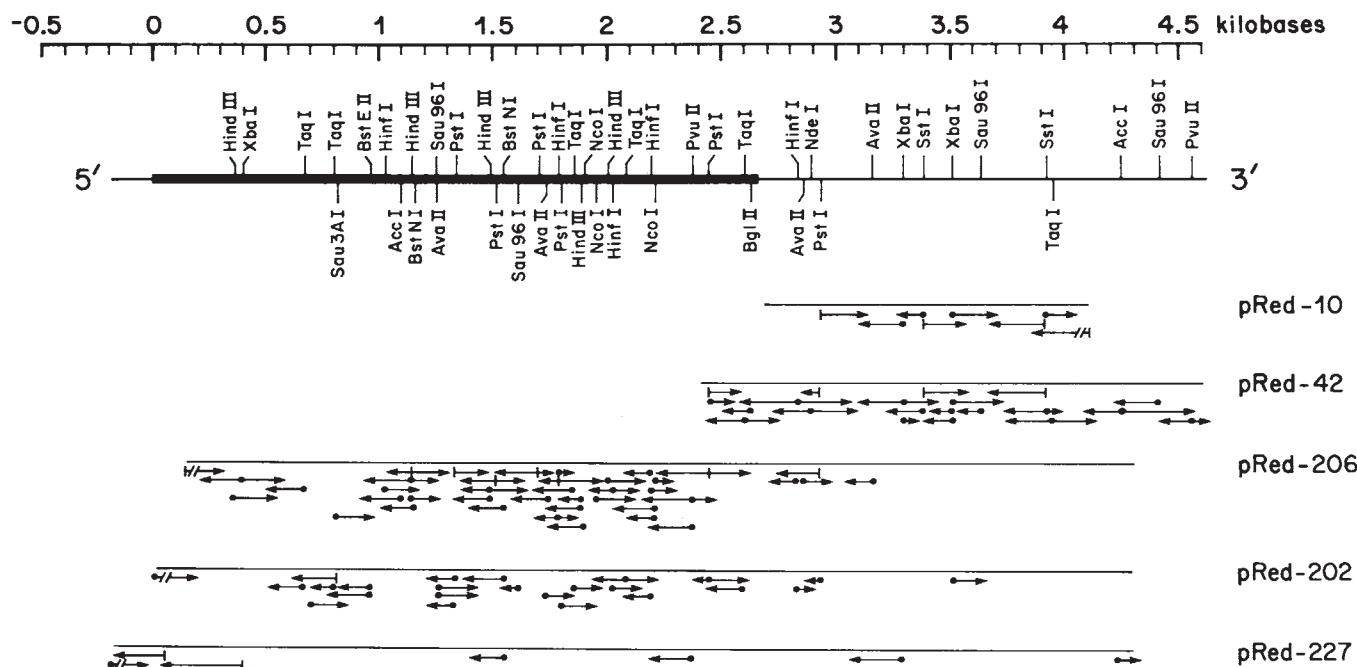


Fig. 1 Restriction map of hamster reductase cDNAs and location of fragments used to determine the nucleotide sequence. The top scale designates the nucleotide positions (in kb) relative to the protein initiator codon beginning at position 1. The restriction map shown in the second line is a composite obtained from the five cDNA inserts. The poly(dA)·poly(dT) and poly(dG)·poly(dC) tails are not shown. The protein coding region is indicated by the thick line. The arrows beneath each cDNA indicate the direction and extent of sequence determination for each fragment analysed. Vertical bars at the ends of the arrows indicate fragments that were subcloned in M13 vectors and sequenced by the dideoxy terminator method⁶; solid circles at the ends of the arrows indicate fragments that were ³²P-labelled at the 3' end and sequenced by the method of Maxam and Gilbert⁷. Double slash marks in certain arrows indicate that the restriction sites used for sequencing or cloning were located in the vector DNA beyond the cDNA insert.

Methods: The five cDNAs were isolated from two libraries. pRed-10 was isolated from library 1 as described elsewhere³. The other four cDNAs were isolated from library 2, which was made with the vectors and methods developed by Okayama and Berg²⁵. To create this library, about 10 µg of poly(A)⁺ mRNA from UT-1 cells were used as a template for cDNA synthesis²⁵. *Escherichia coli* strain χ 1776 cells were transformed with the resultant cDNAs. An aliquot of the χ 1776 library was amplified with chloramphenicol, and plasmid DNA was isolated as described previously²⁶. *E. coli* strain RR1 cells were transformed with this cDNA, and pRed-42 was isolated from the transformants by differential colony hybridization³. pRed-202, -206 and -227 were isolated from a portion of library 2 that was enriched for longer cDNAs by the sub-library method²⁷ and screened with ³²P-labelled 5'-fragments that were chosen to yield increasingly longer cDNAs. For this purpose, each aliquot of 30 µg of total plasmid DNA isolated from the amplified library 2 was digested with 60 U of *Sal*I endonuclease for 1 h at 37 °C in 6 mM Tris-HCl (pH 7.9), 150 mM NaCl, 6 mM MgCl₂, 6 mM 2-mercaptoethanol and subjected to electrophoresis in 1% low gel temperature agarose in 40 mM Tris-HCl (pH 8.2), 20 mM sodium acetate, 20 mM NaCl and 2 mM EDTA at 4 °C. Linearized plasmids with cDNA inserts of 4–6.5 kb were excised from the gel and the DNA was recovered after two extractions each with phenol and with phenol/chloroform (1:1) and one extraction with chloroform. The aqueous phase was adjusted to 2 M ammonium acetate and precipitated with 2 volumes of ethanol; 0.5 µg of the precipitated plasmid DNA was recircularized in a 100 µl reaction containing 100 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 5 mM dithiothreitol, 0.5 mM ATP and 0.2 U T₄ DNA ligase at 14 °C for 15 h. The recircularized DNA was then used to transform CaCl₂-treated *E. coli* strain HB101 cells. Approximately 2–10 × 10³ transformants were obtained from each 30 µg of starting DNA. Colony screening was carried out by standard techniques with ³²P-nick-translated DNA fragments (2 × 10⁵ c.p.m. ml⁻¹; 10⁸ c.p.m. per µg DNA)²⁸. 4 × 10⁴ transformants from the size-fractionated library 2 were screened to obtain pRed-202, -206 and -227.

this class resemble typical plasma membrane and secreted glycoproteins in that they possess hydrophobic NH₂-terminal signal sequences that mediate membrane insertion co-translationally and are then cleaved from the protein. The ER also contains proteins that span the membrane, but lack carbohydrate. Two of these proteins, cytochrome P-450 (ref. 10) and epoxide hydratase¹¹, have been studied indirectly with regard to the presence of cleavable NH₂-terminal signal sequences, and none has been found.

The above data suggest that transmembrane ER proteins that contain carbohydrate also contain cleavable signal sequences, whereas ER proteins that lack carbohydrate also lack cleavable signals. HMG CoA reductase would be an exception to such a rule: it is a glycoprotein, but it lacks a cleavable signal sequence. In the 2,661-bp open reading frame for reductase, the initiator methionine codon is designated as position 1 (Fig. 2). This methionine is retained at the extreme NH₂-terminus of the mature protein as indicated by its relationship to phenylalanine and other methionine residues determined radiochemically (Fig. 3). In this reading frame, two terminator codons, TGA and

TAA, are found 27 and 42 bp, respectively, 5' to the initiator methionine DNA codon. These data suggest that there is no NH₂-terminal extension on the reductase and that translation begins at the methionine designated as position 1 of the cDNA sequence. This finding is in contrast to many cellular and viral transmembrane glycoproteins that have cleaved signal sequences, for example, erythrocyte glycoporphin, heavy chains of membrane-associated IgM, the histocompatibility antigens, the δ subunit of the acetylcholine receptor, G protein of vesicular stomatitis virus, and haemagglutinin of influenza virus¹².

It seems likely that the reductase contains an uncleaved internal signal sequence similar to that which has been reported for ovalbumin (a secreted glycoprotein)¹², the anion transport protein, band III of the erythrocyte (a plasma membrane glycoprotein)¹³, and MP26 of bovine lens fibre (a plasma membrane protein that lacks carbohydrate)^{14,15}. Such internal sequences interact with the signal recognition particle, direct the co-translational insertion of proteins into the ER membrane and are not cleaved from the protein after translocation¹⁵. Consistent with this formulation are the recent findings of Brown and Simoni¹⁶,

[illegible]

Fig. 2 Composite nucleotide sequence of the cDNA corresponding to the mRNA for hamster reductase, and the predicted amino acid sequence of the protein. Nucleotides are numbered (left-hand side) in the 5' to 3' direction. Position 1 corresponds to the first nucleotide of the ATG triplet coding for the initiator methionine. The nucleotides on the 5' side of position 1 are indicated by negative numbers. The predicted amino acid sequence is shown below the nucleotide sequence. Four potential polyadenylation signals in the 3'-untranslated region are underlined. The amino acid residues are numbered (right-hand side) beginning with the initiator methionine. Three potential sites for asparagine-linked glycosylation are boxed. In the coding region, the DNA sequence at nucleotide position 2,018 differed in multiple analyses; T was obtained on three determinations (twice in pRed-202 and once in pRed-206), whereas a G was obtained on two determinations (twice in pRed-206). This single base difference affects the amino acid assignment at position 673 (T→phenylalanine; G→cysteine). In the 3'-untranslated region, four nucleotide discrepancies were noted in the cDNA sequence of pRed-42 and pRed-10. The sequence of pRed-10 contains T, G and A at positions 3,347, 3,586 and 3,792, whereas the sequence of pRed-42 contains C, A and G at these positions. In pRed-10 a single base (T at position 3,887) was deleted compared with pRed-42.

Methods: Plasmids containing reductase cDNAs were propagated in *E. coli* RR1 and HB101 and purified as described previously²⁶. DNA fragments were 3'-end labelled with deoxynucleoside [α -³²P]triphosphates and the Klenow fragment of *E. coli* polymerase I²⁹ or with ³²P-cordycepin and terminal transferase³⁰ and sequenced by the methods of Maxam and Gilbert⁷. Alternatively, appropriate DNA fragments were isolated and cloned into the M13 mp8, mp10 or mp11 vectors³¹⁻³³. Single-stranded DNA from M13 clones was prepared from individual plaques, the inserted DNA fragments were oriented relative to one another³⁴, and single-stranded DNAs of interest were sequenced by the terminator method of Sanger *et al.*⁸, using an M13 universal primer³⁵. For both sequencing methods, gel electrophoresis was performed as described by Sanger and Coulson³⁵. Computer-assisted sequence analysis was accomplished with the programs of IntelliGenetics, Inc.

who showed that hamster HMG CoA reductase is inserted into the ER membrane co-translationally in a signal recognition particle-dependent manner.

The amino acid sequence of the reductase contains three sites that are classic signals for asparagine-linked glycosylation, that is, Asn-X-^{Ser}Thr, where X can be any amino acid except proline (boxes in Fig. 2). Which of the three sites is glycosylated *in vivo* will depend on which sites are exposed on the luminal side of the ER, an as yet unknown orientation.

Untranslated regions

The reductase mRNA contains a 5'-untranslated region of at least 163 bp as indicated by the cDNA sequence (Fig. 2). The actual 5'-untranslated region might be even longer than 163 bp because pRed-227, the longest cDNA, may not extend to the 5' end of the reductase mRNA. A striking feature of this region is the presence of an AUG codon 160 nucleotides upstream from the functional initiation site (ATG at position -160 in the

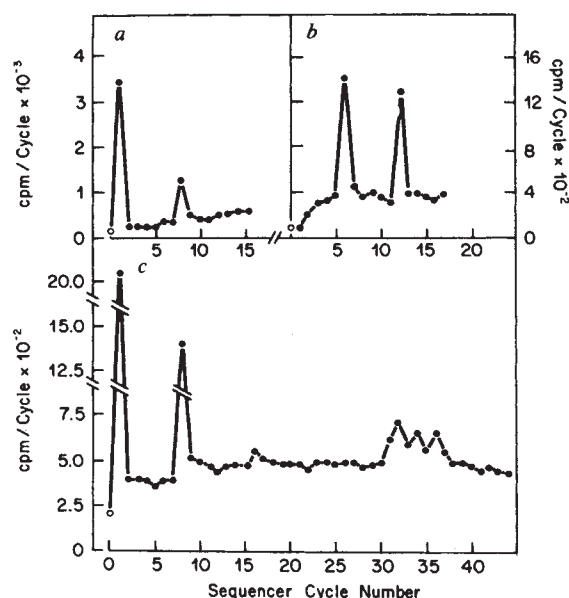


Fig. 3 Partial NH₂-terminal sequence of hamster reductase. UT-1 cells were incubated with either ³⁵S-methionine (a, c) or ³H-phenylalanine (b). The 97,000-M_r reductase was isolated by immunoprecipitation and SDS-gel electrophoresis and taken through 15–43 cycles of Edman degradation. The amounts of radioactivity loaded into the sequencer were as follows: a, 38,000 c.p.m.; b, 291,000 c.p.m.; c, 197,000 c.p.m. The radioactivity released in each cycle is shown.

Methods: UT-1 cells were cultured in 40 μM compactin in Ham's F-12 medium with 10% (v/v) lipoprotein-deficient serum^{2,5}. Cells were incubated for 12 h with either 200 μCi ml⁻¹ of ³⁵S-methionine in methionine-free Ham's F-12 medium or 250 μCi ml⁻¹ of ³H-phenylalanine in minimal Eagle's medium supplemented with all amino acids except phenylalanine. Cells were solubilized with SDS. Reductase was immunoprecipitated with a polyclonal anti-reductase IgG and Pansorbin⁵, eluted from Pansorbin as described previously⁵ and subjected to SDS-polyacrylamide gel electrophoresis⁵. The region of the gel corresponding to a M_r range of 90,000–100,000 was excised, and minced pieces of the gel were soaked in 50 mM NH₄HCO₃ and 0.1% SDS for 15 min at 24 °C, then in 400 mM NH₄HCO₃, 2% SDS, 100 mM dithiothreitol and 1 mg ml⁻¹ bovine serum albumin for 2–4 h. Samples were eluted from the gel in an electrophoretic sample concentrator. Automated amino acid sequence analysis was performed with Beckman 890C or 890D amino acid sequencers using 0.25 M or 0.1 M Quadrol programs³⁶. Before each sequence run, a wash step was performed consisting of an entire cycle without the coupling reagent (cycle 0). The material obtained from each cycle was evaporated to dryness and subjected to scintillation counting.

cDNA sequence of Fig. 2). If this AUG codon initiates translation, protein synthesis would continue for 318 nucleotides to position +158, where the first terminator codon would be encountered (frame A in Fig. 4). This open reading frame overlaps by 157 bp the reading frame for the reductase (frame B in Fig. 4). The putative protein encoded in frame A would contain 106 amino acids with a M_r of 12,212.

The significance of the overlapping open reading frame is unknown. The statistical likelihood that such a 318-bp open reading frame would occur by a random sequence of nucleotides is <0.007. The open reading frame is unlikely to be the result of a cloning artefact (such as a transcription error) or a technical artefact (such as a sequencing error) since an identical sequence from nucleotide positions –163 to +161 was found when the corresponding genomic clones (included in two exons) were sequenced (data not shown). Single mRNAs that contain two functional initiator methionine codons in overlapping reading frames have been described for several viral mRNAs, including adenovirus Elb (ref. 17), bunyavirus s-RNA¹⁸ and influenza B virus segment 6 (ref. 19). In each case the single mRNA species

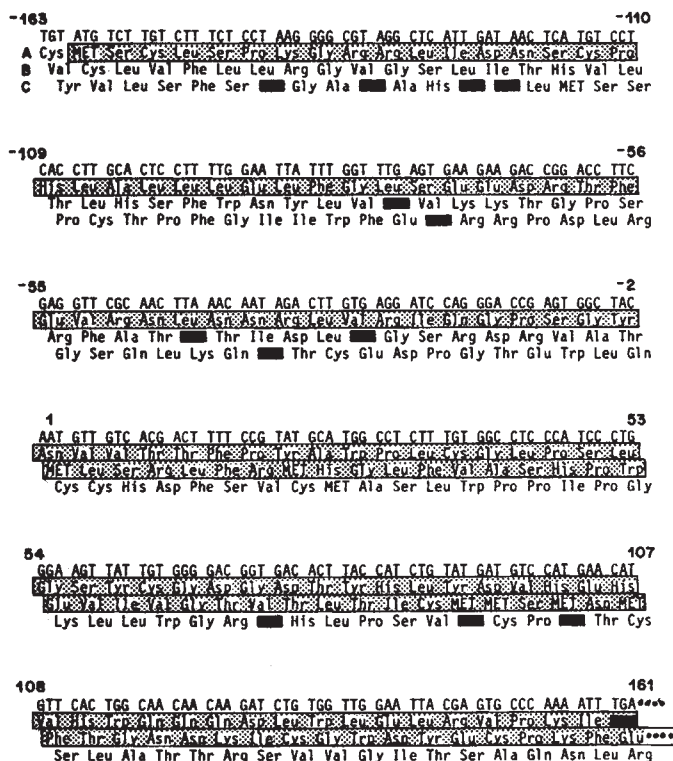


Fig. 4 Nucleotide sequence of the 5' end of reductase cDNA, showing the amino acid sequences encoded by the two overlapping open reading frames, A and B. Frame B, which encodes the reductase, begins at nucleotide position 1 and continues for 887 amino acids. Frame A begins at nucleotide position –160 and continues for 106 amino acids. Terminator codons in the nucleotide sequence are indicated by the black bars.

gives rise to two proteins. Such overlapping bicistronic mRNAs have not been observed in nonviral eukaryotic messages²⁰.

The reductase mRNA contains an unusually long 3'-untranslated region, of 1,942 bp, which does not seem to code for a protein. The longest open reading frame that begins with a methionine codon spans only 174 nucleotides. The length of the 3'-untranslated region has been conserved among different species. Similarly sized 3'-untranslated regions are present in reductase mRNA from cows and rats as well as hamsters, as indicated by similarly sized mRNAs on Northern blots (ref. 21 and our unpublished data). The 3'-untranslated region contains four canonical polyadenylation signals (AATAAA)²², whose positions are shown in Fig. 2, plus a variant signal (AAATTAT) at position 4288. The latter signal appears to be used by mRNAs that gave rise to three cDNAs (pRed-206, -202 and -227, Fig. 1). The relationship between these five polyadenylation signals and the 4.2- and 4.7-kb mRNA observed in UT-1 cells³ is unknown.

Implications

The availability of a near full-length cDNA for reductase should allow definition of sequences that mediate negative feedback regulation. To this end, the genomic sequences of hamster reductase, which span about 25 kb, have been isolated and are now under investigation.

The amino acid sequence of reductase may be a useful model system for studying the structure of proteins of the ER. Plots of the relative hydrophobicity at each amino acid position in the reductase were made according to the Kyte-Doolittle method²³, and revealed seven hydrophobic regions, each of which encompasses at least 20 amino acids and potentially spans the membrane (data not shown). All of these hydrophobic regions are within the NH₂-terminal half of reductase. In contrast, the COOH-terminal half of the reductase is hydrophilic.

Inasmuch as the active portion of reductase can be released from microsomal vesicles proteolytically as a 53,000–55,000-*M_r* water-soluble fragment⁵, it is likely that the catalytic site of the enzyme is present in the hydrophilic COOH-terminal half of the molecule. It is not known why reductase requires such a large hydrophobic NH₂-terminal domain. This membrane-associated domain may have a role in the post-translational regulation of this cholesterol-synthesizing enzyme.

At a more general level, the information obtained about reductase illustrates the power of modern techniques of molecular biology to provide information about large, complex proteins that are of great importance to biology and medicine, yet are difficult to approach through classic techniques of protein chemistry. Amplification of the reductase gene in cultured cells permitted the cDNA cloning of the enzyme and the determina-

tion of its protein sequence before any such information was available through other means. Because of the crucial role of reductase in regulating body cholesterol metabolism²⁴, it seems likely that defects that perturb this regulation may underlie some of the poorly understood hypercholesterolaemic states in man. The studies described here represent a first step in elucidating such defects.

We thank J. Donald Capra and Clive Slaughter for help in the radiochemical sequencing of reductase protein. Clarice Grimes and Edith Womack provided technical assistance in growing UT-1 cells. This work was supported by research grants from the NIH. D.J.C., G.G., L.L. and K.L.L. are recipients of fellowships from the Helen Hay Whitney Foundation, the Juan March Foundation, the NIH and the John A. Hartford Foundation, respectively.

Received 3 November 1983; accepted 11 January 1984.

1. Brown, M. S. & Goldstein, J. L. *J. Lipid Res.* **21**, 505–517 (1980).
2. Chin, D. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 1185–1189 (1982).
3. Chin, D. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 7704–7708 (1982).
4. Luskey, K. L., Faust, J. R., Chin, D. J., Brown, M. S. & Goldstein, J. L. *J. biol. Chem.* **258**, 8462–8469 (1983).
5. Liscum, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 7165–7169 (1983).
6. Anderson, R. G. W., Orci, L., Brown, M. S., Garcia-Segura, L. M. & Goldstein, J. L. *J. Cell Sci.* **63**, 1–20 (1983).
7. Maxam, A. & Gilbert, W. *Meth. Enzym.* **65**, 499–560 (1980).
8. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
9. Kreibich, G., Marcantonio, E. E., Borgese, N. & Sabatini, D. D. *Meth. Enzym.* **96**, 520–530 (1983).
10. Bar-Nun, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 965–969 (1980).
11. Okada, Y. *et al. Eur. J. Biochem.* **122**, 393–402 (1982).
12. Kreil, G. A. *Rev. Biochem.* **50**, 317–348 (1981).
13. Braell, W. A. & Lodish, H. F. *Cell* **28**, 23–31 (1982).
14. Paul, D. L. & Goodenough, D. A. *J. Cell Biol.* **96**, 633–638 (1983).
15. Anderson, D. J., Mostov, K. E. & Blobel, G. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7249–7253 (1983).
16. Brown, D. A. & Simoni, R. D. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
17. Bos, J. L. *et al. Cell* **27**, 121–131 (1981).
18. Bishop, D. H. L., Gould, K. G., Akashi, H. & Clerx-van Haaster, C. M. *Nucleic Acids Res.* **10**, 3703–3713 (1982).
19. Shaw, M. W., Choppin, P. W. & Lamb, R. A. *Proc. natn. Acad. Sci. U.S.A.* **80**, 4879–4883 (1983).
20. Kozak, M. *Microbiol. Rev.* **47**, 1–45 (1983).
21. Liscum, L. *et al. J. biol. Chem.* **258**, 8450–8455 (1983).
22. Nevins, J. R. A. *Rev. Biochem.* **52**, 441–466 (1983).
23. Kyte, J. & Doolittle, R. F. *J. molec. Biol.* **157**, 105–132 (1982).
24. Goldstein, J. L. & Brown, M. S. A. *Rev. Biochem.* **46**, 897–930 (1977).
25. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2**, 161–170 (1982).
26. Clewell, D. B. & Helinski, D. R. *Biochemistry* **9**, 4428–4440 (1970).
27. Okayama, H. & Berg, P. *Molec. cell. Biol.* **3**, 280–289 (1983).
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
29. Smith, M. *et al. Cell* **16**, 753–761 (1979).
30. Roychoudhury, R. & Wu, R. *Meth. Enzym.* **65**, 43–62 (1980).
31. Messing, J., Crea, R. & Seeburg, P. H. *Nucleic Acids Res.* **9**, 309–321 (1981).
32. Vieira, J. & Messing, J. *Gene* **19**, 259–268 (1982).
33. Messing, J. & Vieira, J. *Gene* **19**, 269–276 (1982).
34. Winter, G. & Fields, S. *Nucleic Acids Res.* **8**, 1965–1974 (1980).
35. Sanger, F. & Coulson, A. R. *FEBS Lett.* **87**, 107–110 (1978).
36. Klapper, D. G., Wilde, C. W. III & Capra, J. D. *Analyt. Biochem.* **85**, 126–131 (1978).

LETTERS TO NATURE

Blazars can have double radio sources

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Blazars (BL Lac objects and optically violently variable quasars) are characterized by high optical polarization, and highly variable optical flux, polarization and polarization position angle¹. Many have other extraordinary properties that could be explained by a radiating jet undergoing bulk relativistic motion directed towards the observer. Several workers have suggested that the jets seen in ordinary double radio sources could appear as blazars, if they were viewed end-on¹; in this case the two lobes would not appear separately, but would appear in projection as a halo around the point source. Generally, blazar maps in the literature are consistent with this notion^{2–4}, with only two unusual (bent or very asymmetrical) double sources—1400+162 (refs 3–6) and 3C279 (ref. 7)—having been reported. However, we have now found three clear double radio sources (two being quite symmetrical) around the strong central point sources, including one classical double. A fourth blazar has head–tail morphology. It may be possible to reconcile these observations with the model cited above if we imagine that the jets do not lie precisely along the line of sight, or that the lobes are misaligned with the jets⁵.

The idea that we are looking down a relativistic jet can explain several properties that frequently occur among blazars. Very long baseline interferometry (VLBI) observations have shown motions apparently in excess of the speed of light⁹. Variability time scale upper limits to the size of their compact radio sources

can be smaller than lower limits as inferred from the observed lack of interstellar scintillation¹⁰, and brightness temperatures inferred from fluxes and low radio frequency variability time scales often exceed the theoretical maximum set by comptonization of microwave background photons^{11,12}. Of the 56 blazars recognized at the time of the Angel and Stockman review¹, about 30 have high-resolution, high-dynamic range Very Large Array (VLA) maps in the literature—mainly in refs 2, 3 and 13. We used the VLA in its high resolution (~1.2 arc s) configuration at 20 cm to map the rest of the objects in September 1983.

Unexpectedly, several of our blazars do show double lobes. The hypothesis that blazars are ordinary doubles seen end-on can accommodate some of these if they are borderline objects, with viewing aspects relative to the jet of ~1 or 2 times the beaming angle (γ^{-1} , where γ is the Lorentz factor associated with the bulk motion of the jet). Alternatively, as jets of normal double radio sources are sometimes rather misaligned with the associated lobes (for example, some sources are S-shaped), we know that the jet can be precisely in the line of sight while the lobe axis is not. Both of these possibilities would result in rather unusual-looking doubles, with, for example, large lobe size to separation ratios. Measurements of such properties are sensitive to map quality, so tests must be made with care. A fundamental expectation is that the projected linear sizes should be small. It may be very important that the projected sizes of our doubles are only 50–120 kpc. As indicated below, this is much smaller than typical radio galaxy double sources.

Double radio sources around blazars do not seem to be rare; we have found several, and it is our impression that maps of still higher dynamic range would reveal more. At a dynamic range of ~1,000 to 1 (dynamic range defined as ratio of peak flux to three times the root mean square (r.m.s.) noise), a few sources still show no extended emission.

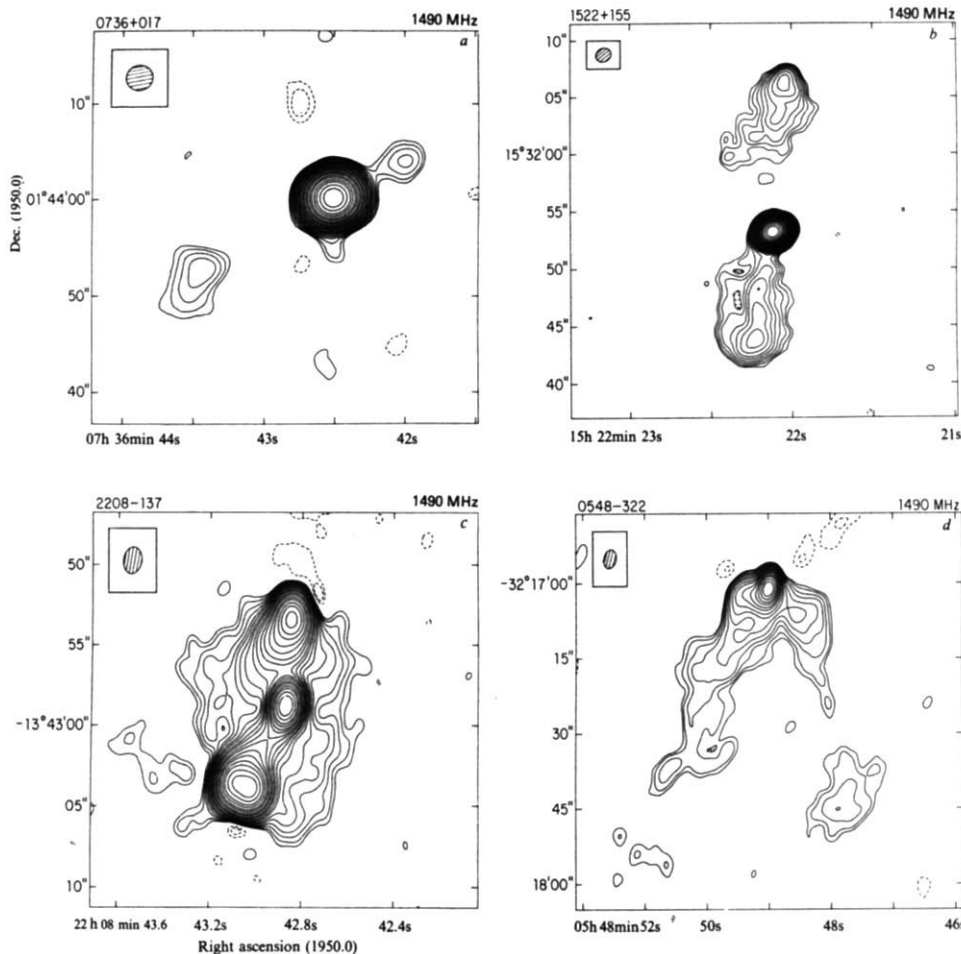


Fig. 1 Radio frequency contour maps of PKS0736+017(a), MC31522+155(b), PKS2208-137(c) and PKS0548-322(d). Contours are logarithmically spaced, with intervals of a factor of $\sqrt{2}$: a, b start with the 0.1% contour, c with the 0.2% contour and d with the 0.8% contour. Peak fluxes are 2.166 Jy, 0.328 Jy, 0.235 Jy, and 0.094 Jy, respectively. The ellipse in the upper left-hand corner of each map shows the half-power beam size. The maps of 0548-322 and 0736+017 were made with 50-k λ gaussian tapers while the others are untapered. As 0736+017 is very near the equator, the map is noisier due north and south of the point source. For that reason the slight southern extension of the point source is not believable.

Here we summarize the relevant properties of the blazars having extended double sources. The extended flux and size values are preliminary, and subject to revision. The measurements do not include any structure which we may have 'resolved out'.

PKS0736+017 (Fig. 1a) has an extended flux density of ~ 35 mJy, corresponding to 2.6×10^{31} erg s $^{-1}$ Hz $^{-1}$ at the redshift of 0.191 (ref. 14). Fanaroff and Riley¹⁵ have shown that normal doubles can be divided into two groups. Class 1 objects are of low luminosity with extended power $P_{1.5\text{GHz}} < 2 \times 10^{32}$ erg s $^{-1}$ Hz $^{-1}$ ($H_0 = 75$ km s $^{-1}$ Mpc $^{-1}$, $q_0 = 1/2$ used throughout). (Fanaroff and Riley included the core luminosities, but these are negligible in normal doubles.) Class 1 objects have relaxed, edge-darkened morphology, whereas the higher luminosity class 2 objects have edge-brightened morphology. The PKS0736+017 extended luminosity places it with the FR class 1 sources. The lobe separation of ~ 28 arc s implies a projected source size of 76 kpc; this compares with a median class 1 intrinsic source size of 150 kpc (ref. 16).

The optical polarization ranges up to 6%, with θ taking on values throughout its 180° range¹⁷. The optical flux has been monitored¹⁸, and it is variable. This object is also a low-frequency radio variable¹⁹.

MC31522+155 (Fig. 1b) is a normal-looking classical (edge-brightened or class 2) double. The extended flux is ~ 70 mJy which, at a redshift of 0.628, corresponds to a power of 6.0×10^{32} erg s $^{-1}$ Hz $^{-1}$, above the cutoff between the FR classes. The reference providing the redshift²⁰ tabulates extraordinary emission line widths and ratios (though their spectrum was very noisy). The ~ 23 arc s extent corresponds to 116 kpc which is rather small compared with other class 2 sources. The median intrinsic class 2 size is 170 kpc and only 10% are smaller than 130 kpc (ref. 16).

The optical light is variably polarized, with $3\% \leq P \leq 13\%$, $10^\circ \leq \theta \leq 105^\circ$ over the course of three observations¹⁷. No

optical or low-frequency radio flux monitoring is available for this double.

PKS2208-137 (Fig. 1c) is a double, probably edge-brightened, embedded in a cocoon of diffuse emission. There is a single emission line at 3,896 Å visible in the spectrum taken by Peterson *et al.*²¹. They believe that the probable identification is Mg II $\lambda 2,798$ at $z = 0.392$, with Ly α at $z = 2.204$ also possible; the extended flux of ~ 820 mJy would correspond to 2.7×10^{33} or 7.1×10^{34} erg s $^{-1}$ Hz $^{-1}$, respectively. In either case this would correspond to a Fanaroff and Riley class 2 object. The angular size is ~ 11 arc s, corresponding to 45 or 57 kpc.

Moore and Stockman¹⁷ have measured the optical polarization four times: both the polarization amount and position angle are variable, and the measurements spanned the range $1\% \leq P \leq 9\%$, $100^\circ \leq \theta \leq 170^\circ$. No optical photometric or low-frequency radio monitoring is available.

PKS0548-322 (Fig. 1d) is the only blazar with head-tail morphology. It has a strange linear structure slightly separated from the eastern tail by an area of low surface brightness. The extended 20-cm flux is ~ 230 mJy. No emission lines are seen but a redshift of 0.069 is available from the stellar absorption features of the host galaxy²². This implies an extended radio power of 2.1×10^{31} erg s $^{-1}$ Hz $^{-1}$. Our map shows that the trail is at least 49 arc s (or 59 kpc) long.

The optical polarization was measured twice, and was found to be low but variable¹. Optical flux variations of one magnitude occur on a time scale of days²³. There are no low-frequency radio flux monitoring data in the literature.

Finally, we note that there is already one case of a radio source with evidence for relativistic motion in the line of sight, but with a normal double radio source. Porcas²⁴ has conducted a VLBI experiment on the core of the quasar 3C 179, specifically because it has normal radio lobes but a core bright enough to observe with Mark II VLBI; though not a known blazar, it does show superluminal motion. (For MERLIN maps of it and other

known superluminal sources, see ref. 7.) Here we have reported the discovery of further normal double sources in objects whose cores show evidence for bulk relativistic motion near the line of sight. Thus, this phenomenon is not as rare as previously supposed.

The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the NSF.

Received 9 December 1983; accepted 7 February 1984.

1. Angel, J. R. P. & Stockman, H. S. *Astr. Astrophys.* **18**, 321–362 (1980).
2. Ulvestad, J. S., Johnston, K. J. & Weiler, K. W. *Astrophys. J.* **266**, 18–27 (1983).
3. Wardle, J. F. C., Moore, R. L. & Angel, J. R. P. *Astrophys. J.* (in the press).
4. Stannard, D. & McIlwraith, B. K. *Nature* **298**, 140–144 (1982).
5. Baldwin, J. A. *et al. Astrophys. J.* **215**, 408–416. (1977).
6. Hintzen, P. & Owen, F. *Astr. J.* **86**, 1577–1584 (1981).
7. Browne, I. W. A. *et al. Nature* **299**, 788–793 (1982).
8. Schilizzi, R. T. & de Bruyn, A. G. *Nature* **303**, 26–31 (1983).
9. Cohen, M. H. & Unwin, S. C. *IAU Symp.* **97**, 345–354 (1982).
10. Condon, J. J. & Dennison, B. *Astrophys. J.* **224**, 835–840 (1978).
11. Condon, J. J., Ledden, J. E., O'Dell, S. L. & Dennison, B. *Astr. J.* **84**, 1–11 (1979).
12. Moore, R. L. in *Proc. of Workshop on Low Frequency Variability of Extragalactic Source* 21–22 April, Green Bank, West Virginia, 49–53 (National Radio Astronomy Observatory, Charlottesville, 1982).
13. Perley, R. A., Fomalont, E. B. & Johnston, K. J. *Astrophys. J. Lett.* **255**, L93–L97 (1982).
14. Baldwin, J. A. *Astrophys. J.* **201**, 26–44 (1975).
15. Fanaroff, B. L. & Riley, J. M. *Mon. Not. R. astr. Soc.* **167**, 31–35 (1974).
16. Miley, G. K. A. *Rev. Astr. Astrophys.* **18**, 165–218 (1980).
17. Moore, R. L. & Stockman, H. S. *Astrophys. J.* **243**, 60–75 (1981).
18. Pica, A. J. *et al. Astr. J.* **85**, 1442–1461 (1980).
19. Spangler, S. R. & Cotton, W. D. *Astr. J.* **86**, 730–746 (1981).
20. Smith, H. E. *et al. Astrophys. J.* **215**, 427–437 (1977).
21. Peterson, B. A., Jauncey, D. L., Wright, A. E. & Condon, J. J. *Astrophys. J. Lett.* **207**, L5–L8 (1976).
22. Fosbury, R. A. E. & Disney, M. J. *Astrophys. J. Lett.* **207**, L75–L77 (1976).
23. Gilmore, G. *Mon. Not. R. astr. Soc.* **190**, 649–667 (1980).
24. Porcas, R. W. *Nature* **294**, 47–49 (1981).

Peculiar radio structure in the quasar 3C380

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The majority of powerful extragalactic radio sources have steep radio spectra and angular sizes >5 arc s. Their basically symmetrical double structures can be explained by means of two, originally antiparallel, beams which emanate from a massive object or central engine (CE) and transport energy to the 'outer lobes' some 10^5 – 10^6 pc away¹. Around one-third of sources, however, are more compact. The flat-spectrum compact sources may be part of the same parent population as the steep-spectrum extended sources but observed end-on rather than broadside-on^{2,3}. As yet, however, we have little understanding of the steep-spectrum compact sources (SSCSs) which have recently been shown to comprise $\sim 30\%$ of all steep-spectrum objects^{4,5}. We now present radio maps which hint at an explanation for the small size of at least some of these SSCSs. Our maps, of the quasar 3C380, reveal a peculiar radio structure which is difficult to interpret other than by a powerful interaction between the radio-emitting plasma and its environment. Thus, the sub-galactic (projected) dimensions of this and other SSCSs may be a result of the beams being disrupted before they have propagated beyond the parent object.

The QSO 3C380 ($z = 0.692$; thus 1 arc s corresponds to 4 kpc for $H_0 = 100$ km s⁻¹, $q_0 = 0.5$) is one of the most powerful radio sources (radio luminosity at 178 MHz, $P_{178} \sim 10^{28}$ W Hz⁻¹) and yet most of its emission at 5 GHz comes from a region only a

few arc s in extent. We have mapped it in detail with the Jodrell Bank MERLIN⁶ and the European very long baseline interferometry network (EVN) using the iterative method described by Cornwell and Wilkinson⁷. Figure 1 (resolution 0.3 arc s) was made from MERLIN data at 1.67 GHz while Fig. 2 (resolution 0.15 arc s) is a composite 1.67-GHz map made by adding EVN data (using telescopes at Jodrell Bank, Dwingeloo and Effelsberg) to the same MERLIN data used to construct Fig. 1. Figure 3 shows the 5-GHz MERLIN map (resolution 0.06 arc s), while the map made from the 1.67-GHz EVN data alone (resolution 0.025 arc s) is shown as an inset to Fig. 3.

Putting together these and other published maps, we now know much more about the radio structure of 3C380 than any other SSCS. In general the most surprising aspect of the structure is its overall complexity (see below) but despite this there is a recognizable axis, in position angle (p.a.) $\sim -52^\circ$, which is constant to $\pm 5^\circ$ over a range in spatial scale of several hundred to one. This can be traced through the brightest component, or 'nucleus', on scales of ~ 0.015 arc s (refs 8, 9) and ~ 0.050 arc s (Fig. 3), through two bright 'knots' about 1 arc s away (Figs 2, 3) and finally through the weak 'jet' to the north-west (Fig. 1). The end of this weak north-west jet then turns abruptly to the north-east. In detail the most curious aspect of the structure is the two, roughly parallel, curved features which seem to emerge from the nucleus and the compact knots (Fig. 2). Either or both of these features may well be associated with the long thin feature ~ 2 arc s east of the nucleus (Fig. 1), thus constituting an 'arc' of emission which apparently bends through $\sim 160^\circ$. Finally there is amorphous low brightness emission around the source whose maximum extent is ~ 8 arc s (Fig. 1 and ref. 10); a Westerbork map at 609 MHz with a dynamic range $>10,000:1$ shows no evidence for any significantly more extended emission (G. de Bruyn and P.N.W., unpublished).

Using other unpublished data, we have obtained rough information about the spectra of these various regions. All the emission, apart from that from the nucleus, appears to be optically thin down to $\nu \leq 400$ MHz, with a spectral index, α , within ~ 0.2 of the integrated $\alpha \sim 0.7 (S \propto \nu^\alpha)$. The nuclear spectrum on the other hand, clearly shows optical depth effects from 5 GHz (nucleus contributes 40% of the total flux) to ≤ 400 MHz (nucleus contributes $\sim 10\%$ of the total flux) with an ill-defined peak at ~ 1 GHz. The peculiar structure of the steep-spectrum emission means that 3C380 violates the well-established correlation between monochromatic luminosity and radio structure for extended steep-spectrum radio sources¹¹; powerful radio galaxies and QSOs ($P_{178} > 10^{26}$ W Hz⁻¹) are usually edge-brightened doubles while it is the weaker radio galaxies which have more complex structures¹².

Why does this high luminosity source have this peculiar shape? While the strong, elongated, milliarc s nucleus is good evidence that at least part of the tangle of emission is associated with material ejected from a CE, we have considered the (unlikely) possibility that 3C380 is a chance superposition of two unrelated sources. The evidence is against this, however. For example, while the shape of the arc resembles parts of very low-luminosity systems, such as the anomalous radio continuum arms in the nearby spiral galaxy NGC4258 (ref. 13), the brightness temperatures in these arms are three to four orders of magnitude lower than in the arc. Condon *et al.*¹⁴ have observed more intense amorphous emission, probably due to multiple supernova remnants, in the central regions of some spirals, but again the brightness temperatures are too low (by at least an order of magnitude) and even so, such a spiral could not have been missed on optical plates. If, on the other hand, the second source were a radio galaxy or quasar, its presence would be betrayed by a second CE and hence a second milliarc s nucleus. The nucleus contains structure on the milliarc s scale^{8,9} and so the turnover in its radio spectrum at ~ 1 GHz is very probably due to synchrotron self-absorption: however, the compact knot, which is the obvious place to look for a second CE, does not show self-absorption and so by implication it has no milliarc s structure and therefore is not the site of a second CE.

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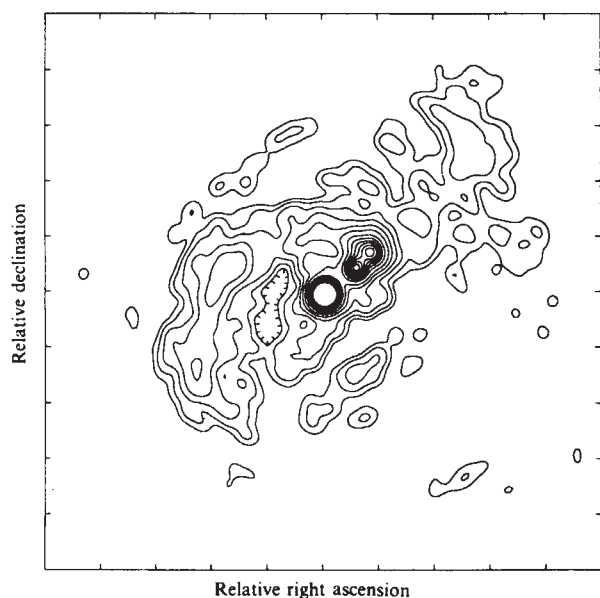


Fig. 1 MERLIN maps at 1,660 MHz with a resolution (FWHM) of 0.3 arc s; contour levels 0.075, 0.15, 0.3, 0.6, 1.25, 2.5, 5, 7.5, 10, ... 25% of the peak brightness of 3.53 Jy per beam. The scale corresponds to 1 arc s per tick.

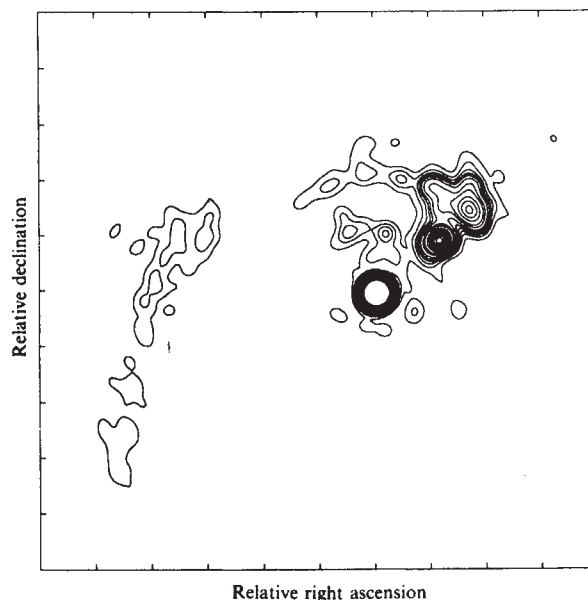


Fig. 2 MERLIN maps at 1,660 MHz showing the brightest features with a resolution of 0.15 arc s; contour levels 0.3, 0.6, 0.9, 1.2, 1.5, 2, 3, 4, 6, 8, 10, ... 20% of the peak brightness of 3.27 Jy per beam. The scale corresponds to 0.5 arc s per tick.

The so-called jets in radio maps of powerful sources are assumed to delineate the paths of beams emanating from the CE. However, two jets are not always visible and jets can appear to be bent for a variety of reasons, thus simple twin-beam models can successfully explain the structures of many morphologically peculiar radio galaxies. We do not think that they can explain the structure of 3C380, however. It is by no means obvious from the present maps where the beams are although it is plausible that the axis in p.a. $\sim -52^\circ$ does mark one beam path because then the knots, and particularly the brightening of the closer, compact (~ 0.040 arc s) knot on the side facing the nucleus (Fig. 3, inset) can be interpreted¹⁵ as resulting from obstructions in the flow.

Many variations on the twin-beam theme may be tried, depending where one thinks a second beam could be located. For example if the beams are relativistic and the source axis is close to the line of sight, emission from the approaching beam is amplified while the second, receding beam can become too weak to be detected. Could 3C380 therefore be a powerful double source viewed from a fortuitous direction with its diffuse emission being the outer lobes projected against each other? We believe not, although we cannot be certain at this stage. The curious curved features must be accounted for and although the hot spots and ridges in the lobes of Cygnus A¹⁶, for example, could give rise to strange radio structures in projection 3C380 does not look like a superposition of separate components. Also, to reduce the apparent size of a typical powerful double (~ 170 kpc, ref. 12) to that of 3C380 (30–35 kpc), the source axis must lie $\leq 15^\circ$ of the line of sight. The *a priori* probability of this is $\sim 1\%$ and so while an individual object could be 'interpreted' like this, the great majority of powerful SSCs cannot be due simply to projection effects.

Alternatively one could imagine that the arc marks the path of a second beam which originates in the nucleus and thus a twisted twin-beam structure is apparently produced. Such structures can be produced if the axis of the CE is precessing, the beam velocities are relativistic and their trajectories are ballistic (that is, unaffected by their environment). As Gower *et al.*¹⁷ have illustrated, the beams can then appear to start from the nucleus in very different directions due to a combination of projection and light travel time effects. However, since rotation symmetry cannot be destroyed by projection, the end of the faint north-west jet should bend to the south-west rather than the north-east as observed. Also, with this type of model one

cannot account simultaneously for the curved feature associated with the north-west knot complex and the north-west jet itself. While we have not attempted any detailed modelling of relativistic precessing beams, these dichotomies convince us that motion of the radio plasma in 3C380 cannot be purely ballistic. We conclude that simple twin-beam models do not naturally account for the shape of 3C380 and that, as a corollary, the physical conditions in the source may well be unusual.

There is increasing evidence that in nearby active galaxies the energy-carrying beams can be bent and braked by interactions with dense interstellar material as they propagate through the body of the galaxy^{18–20}. The complicated shape of 3C380—and particularly the structure of the north-west knot complex (see Figs 2, 3) which looks as if it could be a result of material impinging from the south-west—leads us to think that a plasma/gas interaction may also be the underlying causative agent even in this highly luminous source. Its projected size is somewhat larger—but still of sub-galactic dimensions—and the minimum plasma pressures inferred for the extended structure ($\sim 10^{-8.5}$ dyn cm⁻²) are not very much greater than those in the nearby galaxies^{19,20}. However, such an interaction would have to be considerably stronger than in these nearby galaxies, for they have less distorted radio structures than has 3C380.

van Breugel *et al.*²¹ have independently suggested that other powerful SSCs are objects in which the beams are interacting with dense interstellar gas, citing as evidence their low radio polarization and distorted radio structures. While none of their radio maps show anything like the detail—and hence the degree of distortion—visible in the present maps, we did not measure the polarization distribution and also there is little information from optical data about the thermal gas in 3C380. The optical spectrum does have quite strong narrow emission lines but the high excitation line [OIII] $\lambda 5,007$ —often indicative of extended gas²²—is redshifted to $\lambda 8,470$ and is difficult to observe (B. and D. Wills, personal communication).

One can, however, make a fairly persuasive circumstantial case that gas with the right properties will be found in 3C380. We infer that this gas will have a higher energy density than that in the nearby galaxies in which beam/gas interactions have been studied and the likeliest place to find such gas is in violent galactic mergers involving gas-rich systems. There is increasing evidence that quasars are associated with such systems^{22,23}; the clearest example is 0351+026, a pair of gas-rich dwarf galaxies²⁴ in which the velocity dispersion of the extended gas is very

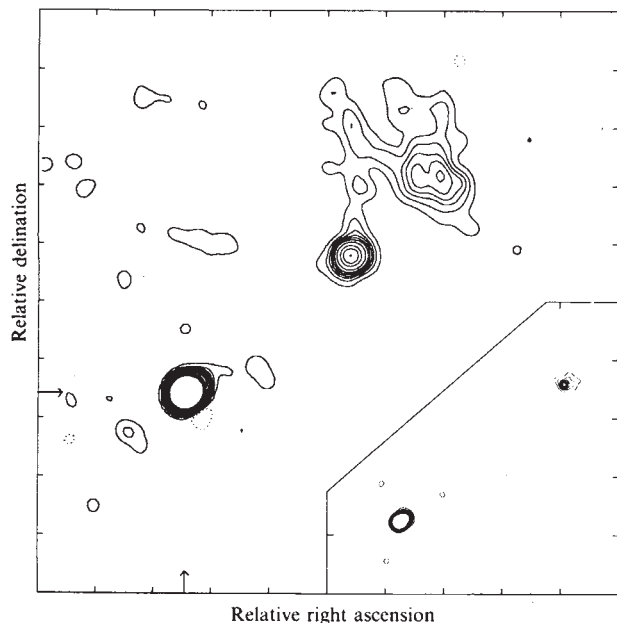


Fig. 3 MERLIN map at 5 GHz showing the structure of the north-west knot complex with a resolution of 0.06 arc s; contour levels 0.2, 0.5, 0.8, 1.1, 1.4, 1.7, 2, 3, 4, ... 10% of the peak brightness of 2.11 Jy per beam. The scale corresponds to 0.2 arc s per tick. Inset: EVN map at 1,660 MHz, showing the nucleus and the brightest knot only with a resolution of 0.025 arc s; contour levels 1, 2, 3, 4, 5, ... 10% of the peak brightness of 2.28 Jy per beam.

high (up to $\sim 1,000 \text{ km s}^{-1}$). 0351+026 bears a 'striking resemblance'²⁵ to the quasar 3C48 and this is an important pointer because, like 3C380, 3C48 is a very powerful SSCS with an odd radio structure^{21,26}. The galactic-sized nebula associated with 3C48 also contains emission-line gas^{25,27} moving at high velocity ($\sim 500 \text{ km s}^{-1}$) with respect to the underlying galaxy, which has the spectral characteristics of a spiral²⁷, that is, a gas-rich object.

Extended gas with the right properties to disturb the beams more strongly than in active galaxies has therefore been directly observed in QSOs. In this context it is worth noting the apparent distinction between powerful SSCSs which are identified with QSOs and those identified with galaxies. Recent MERLIN and EVN maps show that the QSOs more often contain radio sources with peculiar structures while the structures of the galaxies are usually more linear²⁶. We feel that this evidence points towards a relationship between the QSO phenomenon and the distortions visible in many powerful SSCSs—of which 3C380 is currently the best studied example. It is plausible that interstellar gas, highly energized as a result of a galaxy merger, forms part of the link.

Finally, Fig. 1 suggests to us that rotation has had a major role in the recent history of 3C380. If we then speculate that both the curious curving features associated with the nucleus and the compact knots (Fig. 2) are coeval and were produced in the same way, then the centre of rotation cannot be at the nucleus but must be offset to the south-east by ~ 1.5 arc s. This is consistent with the galaxy merger hypothesis if one of the galaxies is radio quiet while the other contains the radio source. The two galaxies will orbit their mutual centre of mass and so the centre of rotation will not be at the radio nucleus.

These first really detailed maps of a powerful SSCSs, the quasar 3C380, reveal an unexpected structure for a source of this luminosity but we cannot explain why the radio emitting plasma is distributed in this odd way. A circumstantial case can be made that the energy-carrying beams are interacting with energetic interstellar gas resulting from a galaxy merger, but the merger hypothesis cannot be proven until we have a better optical image of the QSO, perhaps from the Space Telescope.

Nevertheless, in the more immediate future radio polarization maps should give us a good idea of whether or not there is sufficient ionized gas around the radio components for the gas interaction hypothesis to be tenable.

We thank many colleagues for the efficient operation of MERLIN and the EVN, Bev and Derek Wills and Richard Simon for permission to quote unpublished results, Ger de Bruyn for the Westerbork data reduction and Robin Conway, Ian Browne and Colin Lonsdale for helpful discussions. P.N.W. acknowledges receipt of an SERC Advanced Fellowship.

Received 16 January; accepted 10 February 1984.

1. Rees, M. J. *IAU Symp.* No. 97, 211–222 (1982).
2. Blandford, R. D. & Konigl, A. *Astrophys. J.* **232**, 34–48 (1979).
3. Orr, M. & Browne, I. W. A. *Mon. Not. R. astr. Soc.* **200**, 1067–1080 (1982).
4. Kapahi, V. K. *Astr. Astrophys. Suppl.* **43**, 381–393 (1981).
5. Peacock, J. & Wall, J. V. *Mon. Not. R. astr. Soc.* **198**, 843–860 (1982).
6. Davies, J. G., Anderson, B. & Morison, I. *Nature* **288**, 64–66 (1980).
7. Cornwell, T. J. & Wilkinson, P. N. *Mon. Not. R. astr. Soc.* **196**, 1067–1086 (1981).
8. Readhead, A. C. S. & Wilkinson, P. N. *Astrophys. J.* **235**, 11–17 (1980).
9. Pearson, T. J. & Readhead, A. C. S. *Astrophys. J.* **248**, 61–81 (1981).
10. Hartas, J. S., Rees, W. G., Scott, P. F. & Duffett-Smith, P. J. *Mon. Not. R. astr. Soc.* **205**, 625–636 (1983).
11. Fanaroff, B. L. & Riley, J. M. *Mon. Not. R. astr. Soc.* **167**, 31P–35P (1974).
12. Miley, G. K. *A. Rev. Astr. Astrophys.* **18**, 165–218 (1980).
13. van Albada, G. D. & van der Hulst, J. M. *Astr. Astrophys.* **115**, 263–269 (1982).
14. Condon, J. J., Condon, M. A., Gistler, G. & Puschell, J. J. *Astrophys. J.* **252**, 102–124 (1982).
15. Blandford, R. D. & Konigl, A. *Astrophys. Lett.* **20**, 15–21 (1979).
16. Hargrave, P. J. & Ryle, M. *Mon. Not. R. astr. Soc.* **166**, 305–307 (1974).
17. Gower, A., Gregory, P. C., Hutchings, J. B. & Unruh, W. G. *Astrophys. J.* **262**, 478–496 (1982).
18. Wilson, A. S. & Ulvestad, J. S. *Astrophys. J.* **236**, 576–594 (1982).
19. Heckman, T. M., Miley, G. K., Balick, B., van Breugel, W. J. M. & Butcher, H. R. *Astrophys. J.* **262**, 529–553 (1983).
20. van Breugel, W. J. M., Heckman, T. M., Butcher, H. R. & Miley, G. K. *Astrophys. J.* **277**, 82–91 (1984).
21. van Breugel, W. J. M., Miley, G. K. & Heckman, T. M. *Astr. J.* **89**, 5–22 (1984).
22. Stockton, A. & MacKenty, J. W. *Nature* **305**, 678–682 (1983).
23. Hutchings, J. B. & Campbell, B. *Nature* **303**, 584–588 (1983).
24. Bothun, G. D. *et al. Astr. J.* **87**, 1621–1627 (1982).
25. Balick, B. & Heckman, T. M. *Astrophys. J.* **265**, L1–L5 (1983).
26. Wilkinson, P. N., Spencer, R. E., Readhead, A. C. S. & Simon, R. S. *IAU Symp.* No. 110 (in the press).
27. Boroson, T. A. & Oke, J. B. *Nature* **296**, 397–399 (1982).

A new model for the role of the oceans in determining atmospheric P_{CO_2}

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Recent ice-core measurements have revealed that the atmospheric CO_2 level increased comparatively rapidly by about 70 p.p.m. at the end of the last ice age¹. Here we present an ocean-atmosphere model in which changes in the productivity of high latitude surface waters (from which deep water is formed and circulated around the world's ocean) and/or in the thermohaline overturning rate can lead to substantial changes in atmospheric partial pressure of carbon dioxide (P_{CO_2}), over a concentration range 163–425 p.p.m. A major contribution to the low P_{CO_2} of the last ice age may have been an increase in the net high latitude productivity, possibly coupled with a decrease in the thermohaline overturning.

Figure 1 shows that three-quarters of the ocean volume, approximately everything below 1 km depth, interacts with the atmosphere through $\leq 4\%$ of the ocean's high latitude surface area. The time scale of this interaction is of the order of 1,000 yr (ref. 2). An additional 16% of the ocean volume interacts with the atmosphere through 16% of the surface area on a time scale of the order of 10–100 yr (ref. 3). The processes occurring in these very small areas of contact between the deep ocean and the atmosphere are very important because of the large volume of ocean involved.

GEOSECS and TTO data show that freshly formed deep waters, those most recently in contact with the atmosphere, have P_{CO_2} and P_{O_2} approximately equal to atmospheric levels, and nutrient concentration levels between those of deep water and depleted low latitude surface water. Most simple models of the ocean-atmosphere system, in which the contact between the deep ocean and atmosphere is represented either through a surface box of nutrient-depleted low latitude water⁴⁻⁶ or by direct contact^{6,7} are inconsistent with these observations. In the latter case, the nutrient concentrations at the deep water outcrop will be those of deep water, far higher than observed. More significantly, the P_{O_2} and P_{CO_2} will be those of deep water, much too low for O_2 and too high for CO_2 .

Figure 2 shows our 4-box model of the ocean-atmosphere. It differs from most other ocean box models in that the deep water contacts the atmosphere predominantly through a high latitude box that has properties somewhere between those of nutrient-depleted low latitude surface waters and nutrient-enriched deep waters. The addition of a high latitude box in our model makes it possible to form deep water with appropriate characteristics.

We will discuss the solution of the equations for the 4-box model elsewhere (J.R.T. and J.L.S., in preparation). An excellent approximation can be obtained by leaving out the intra-ocean fluxes from low latitude surface water, f_{ld} and f_{lh} . The reduction in the number of variables allows us to use the carbon-14 distribution in combination with the PO_4 , AOU (apparent oxygen utilization, used instead of O_2), ΣCO_2 , and alkalinity (ALK), to place strong constraints on the magnitude of the overturning, T and f_{hd} (see Fig. 2 legend for conventions).

We first discuss a solution of our box model for the modern ocean. The particle fluxes p_l and p_h (l refers to low latitude and h to high latitude) into the deep ocean are obtained in terms of PO_4 so that PO_4 is our master variable, although one could as easily use NO_3 . Zero PO_4 concentration is assumed in the low latitude surface box:

$$p_l = TPO_{4d} \quad (1)$$

$$p_h = PO_{4d}f_{hd} - PO_{4h}(f_{hd} + T) \quad (2)$$

The conservation equations for AOU, ΣCO_2 and ALK in the deep box then give:

$$PO_{4h} = PO_{4d} - (AOU_d)/r_{O_2/PO_4} \quad (3)$$

$$\Sigma CO_{2h} = \Sigma CO_{2d} - r_{C/PO_4}(PO_{4d} - PO_{4h}) \quad (4)$$

$$ALK_h = ALK_d - r_{ALK/PO_4}(PO_{4d} - PO_{4h}) \quad (5)$$

r_{O_2/PO_4} is the Redfield ratio of O_2 needed to completely oxidize organic matter. We use $r_{O_2/PO_4} = 136$ (refs 5, 8) and r_{C/PO_4} ,

which includes the carbon in $CaCO_3$, is 131 (refs 5, 8). r_{ALK/PO_4} is 38 (refs 5, 8).

We have assumed that $AOU_h = 0$, that is, oxygen is in atmospheric equilibrium. We use equation (3) to find PO_{4h} from measurements of PO_{4d} and AOU_d . If $AOU_h > 0$, as may be the case where deep water is forming very rapidly, PO_{4h} would be slightly larger and our results would have to be modified accordingly. Equations (4) and (5) are used to obtain estimates of ΣCO_{2h} and ALK_h from measured estimates of ΣCO_{2d} and ALK_d .

The balance of alkalinity and carbon in the low latitude surface box, and of carbon dioxide in the atmosphere gives us:

$$ALK_l = ALK_d - r_{ALK/PO_4}(PO_{4d}) \quad (6)$$

$$\Sigma CO_{2l} = \Sigma CO_{2d} - r_{C/PO_4}(PO_{4d}) + f_{al}\beta(\theta_l)(P_{CO_{2a}} - P_{CO_{2l}})/T \quad (7)$$

$$\beta(\theta_h)f_{ah}(P_{CO_{2a}} - P_{CO_{2h}}) = \beta(\theta_l)f_{al}(P_{CO_{2a}} - P_{CO_{2l}}) \quad (8)$$

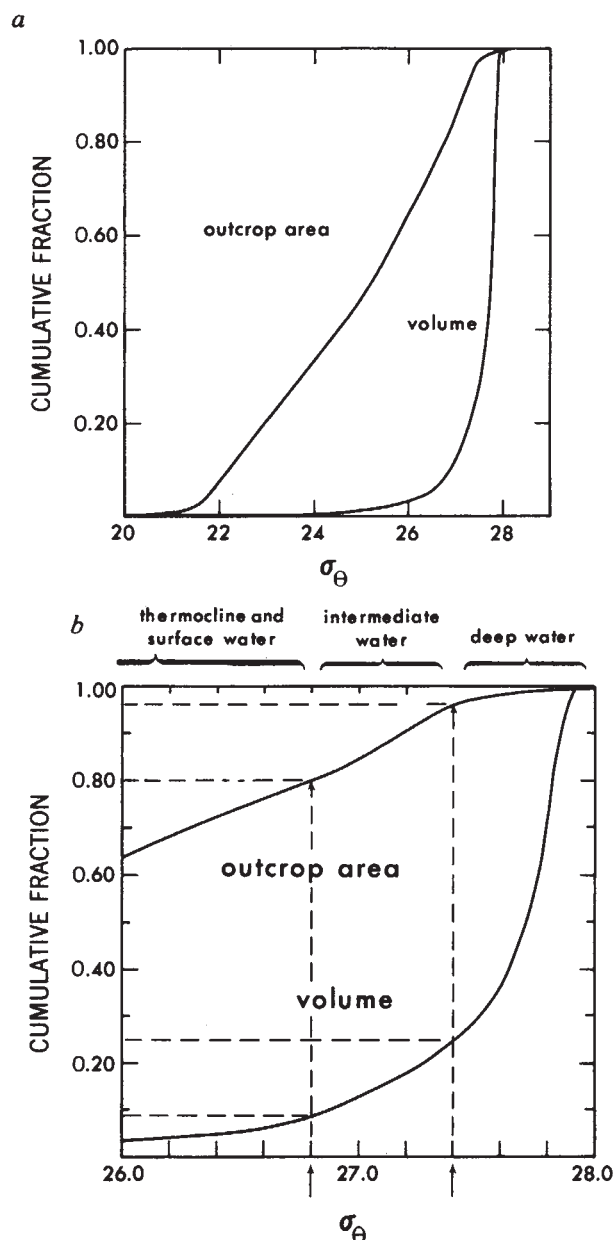


Fig. 1 NODC hydrographic data mapped by Levitus¹⁴ for the three winter months have been used to calculate surface outcrop areas and interior volumes contained in σ_θ intervals of 0.1. σ_θ is potential density referred to the surface. The figure shows the cumulative fraction of these two quantities. For example, 75% of the ocean volume has a $\sigma_\theta > 27.4$. Water in this density range outcrops in <4% of the surface ocean.

Table 1 Model predictions

		Present pre-industrial	Ice age scenario
Model parameters	p_h (mol Cs ⁻¹)	2.31×10^6 *	11.48×10^6
	f_{hd} (m ³ s ⁻¹)	38.1×10^6	38.1×10^6
	T (m ³ s ⁻¹)	25.4×10^6	9.0×10^6
Atmosphere	P_{CO_2} (p.p.m.)	265	200
High latitude surface ocean	PO_4 (μ M kg ⁻¹)	1.02	0
	ΣCO_2 (μ M kg ⁻¹)	2,121	2,213
	ALK (μ equiv. kg ⁻¹)	2,329	2,517
	P_{CO_2} (p.p.m.)	259	185
Low latitude surface ocean	ΣCO_2 (μ M kg ⁻¹)	1,941	2,044
	ALK (μ equiv. kg ⁻¹)	2,290	2,517
	P_{CO_2} (p.p.m.)	269	205
Deep ocean	PO_4 (μ M kg ⁻¹)	2.15	2.24
	AOU† (μ M kg ⁻¹)	154	305
	ΣCO_2 (μ M kg ⁻¹)	2,269	2,506
	ALK (μ equiv. kg ⁻¹)	2,372	2,601

* This flux is equivalent to $1.39 \text{ mol C m}^{-2} \text{ yr}^{-1}$.

† Deep ocean $O_2 + AOU \sim 320 \mu\text{M kg}^{-1}$.

The β values are solubilities of CO_2 in waters of temperature $\theta_l = 21.5^\circ\text{C}$, and $\theta_h = 2.5^\circ\text{C}$ respectively. The solubilities are obtained from the equations of Takahashi *et al.*⁹, as are P_{CO_2l} and P_{CO_2h} . The latter are functions of the temperature, salinity (we use 34.7‰ for the present ocean), alkalinity and total carbon. We have used Broecker and Peng's⁴ estimates of gas exchange rates from radon-222 measurements to obtain $f_{ah} = 3 \text{ m day}^{-1} \times 0.15 \times (\text{ocean area})$ and $f_{al} = 3 \text{ m day}^{-1} \times 0.85 \times (\text{ocean area})$.

We now have eight equations, 10 if we include the Takahashi equations for P_{CO_2l} and P_{CO_2h} , with 15 'unknowns'. We use GEOSECS and TTO data to find PO_{4d} , AOU_d and ALK_d . ΣCO_{2d} can also be estimated from data, but it includes a fossil fuel component, so we prefer to specify $P_{\text{CO}_2d} = 265 \text{ p.p.m.}$ (ref. 1) and calculate ΣCO_2 from the data. T and f_{hd} , which do not enter into equations (1) to (8), are obtained by consideration of radiocarbon observations (J.R.T. and J.L.S., in preparation).

The first column of Table 1 gives our best estimates of the present pre-industrial model variables. Our predicted value for the deep ocean ΣCO_2 , $2,269 \mu\text{m kg}^{-1}$, compares very well with our estimate of $2,274 \mu\text{m kg}^{-1}$ from GEOSECS and TTO data.

We now turn to a consideration of how the ocean and atmosphere change in response to changes in the physical processes, f_{hd} and T , and the high latitude productivity, p_h . We find that the results are very sensitive to whether or not one conserves total tracer in the ocean-atmosphere system. We thus add three new equations to our model: conservation of total alkalinity, carbon and phosphate. This also adds three new 'unknowns', ALK_t , ΣCO_{2t} and PO_{4t} , where t refers to total. We now have 13 equations and 19 'unknowns', including now f_{hd} in the unknowns. We solve the equations by specifying the total alkalinity, carbon and phosphate, as calculated from present values in Table 1. f_{hd} , T and p_h are also specified as some fraction of the present ocean values.

Because of the way we have set up our equations, using phosphate as a master variable, the parameters f_{hd} , T and p_h enter into only three of them: equations (1), (2) and (7). If we

substitute equation (2) into the total phosphate conservation equation, we arrive at:

$$\text{PO}_{4h} = (M_t \text{PO}_{4t} - M_d p_h / f_{hd}) / [M_h + M_d(1 + T/f_{hd})] \quad (9)$$

where M is the mass of a given reservoir in kg.

Consideration of this equation in combination with equation (7) reveals the role of f_{hd} , T and p_h . Consider, first, the high latitude particulate flux, p_h . If this is decreased to zero, the high latitude phosphate will reach a maximum value, with ΣCO_{2h} and ALK_h following suit. The P_{CO_2h} will thus also be at its maximum value. Conversely, an increase of p_h , because of its effect on PO_{4h} , will lead to a decrease in PO_{4h} , ΣCO_{2h} , ALK_h and P_{CO_2h} , with an absolute minimum reached when PO_{4h} is zero.

However, the atmospheric P_{CO_2} depends on the low latitude P_{CO_2} as well as the high latitude P_{CO_2} (equation (8)), and we must thus consider equation (7) as well. The sensitivity of low latitude ΣCO_2 and P_{CO_2} to the high latitude processes depends on the magnitude of T . If T is very large, that is, if the low latitude surface waters are being resupplied very rapidly from deep waters, the low latitude waters will be relatively insensitive to high latitude processes. A small T will mean that the low latitude carbon concentration will be determined primarily by the atmosphere, which will in turn be dominated by high latitude waters.

The effect of variations in f_{hd} can be seen from equation (9); f_{hd} supplies nutrients to the high latitude waters. An increase in f_{hd} increases PO_{4h} , a decrease in f_{hd} decreases PO_{4h} .

The arguments given above are illustrated with the solutions in Fig. 3. In Fig. 3a, f_{hd} is fixed and p_h and T are varied about the values p_h° and T° taken from the present pre-industrial column of Table 1. For large T , the atmospheric P_{CO_2} approaches a value of 250 p.p.m. and is not sensitive to p_h . The sensitivity of atmospheric P_{CO_2} to p_h increases greatly as T is reduced.

In Fig. 3b, p_h is fixed and f_{hd} and T are varied about the values f_{hd}° and T° . One sees that the effect of f_{hd} on P_{CO_2} is essentially the inverse of the effect of p_h on P_{CO_2} .

Figure 3c shows a possible range of scenarios for the last ice age. We have fixed f_{hd} and varied p_h , but one would obtain the same effect by varying f_{hd} . It can be seen from Fig. 3c that a decrease of atmospheric P_{CO_2} to 200 p.p.m. (ref. 9) requires a rather extreme increase in p_h (decrease of f_{hd}) of the order of 4–5 times, accompanied by about a halving of T . The total ocean volume of our ice age model has been decreased by 3.5% to account for glacier formation. The total carbon and alkalinity have been increased by 6% to account for the shift in $\delta^{13}\text{C}$ and a compensatory gain of CaCO_3 from the sediments as postulated by Broecker⁵. This increase in carbon and alkalinity makes it more difficult to achieve the lower P_{CO_2} found for the last ice age.

The work of Boyle and others gives evidence of a drop in North Atlantic deep water formation during the last ice age^{10,11}. This would translate into a drop in f_{hd} and/or T in our model. A decrease in f_{hd} would be helpful. A decrease in T would be helpful, if accompanied by a sufficient increase in productivity or decrease in f_{hd} . A likely cause of changes in surface production is a change in the light supply to the subpolar gyres. Organism growth in these areas is presently light-limited. The gyres straddle the present polar night line ($\sim 60^\circ\text{N}$). A shift in the deep water formation regions to lower latitudes by sea ice blockage of higher latitudes would drive the water to regions where organisms would have a more ample light supply. We consider it unlikely that p_h would increase sufficiently to explain the entire decrease in P_{CO_2} . A decrease in f_{hd} would help.

A critical test of the various models for predicting ice age P_{CO_2} is the extent to which they can give the observed rapid time scales of the change. Our model allows us to develop a scenario involving only a change in p_h and T , which would adjust on a time scale of the order of 1,000 yr. This is consistent with the data¹. The scenario in Fig. 3c as well as Broecker's⁵, would take about 5,000 yr because of the need to readjust the CaCO_3 balance in the oceans. Five thousand years is longer than the data allow. The increase in oceanic carbon required by the $\delta^{13}\text{C}$

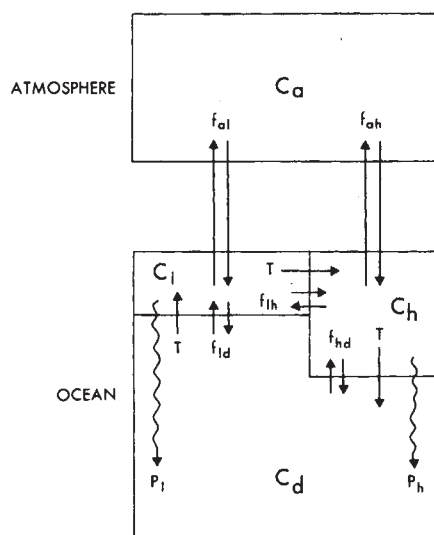


Fig. 2 Schematic diagram of our 4-box model. f , Exchange rates in units of $\text{m}^3 \text{s}^{-1}$. Subscripts: a, atmosphere; l, low latitude nutrient-depleted surface water; h, high latitude deep water formation regions; d, deep ocean. In addition to exchange rates, the model allows for a shallow thermohaline overturning, $T (\text{m}^3 \text{s}^{-1})$, which goes through the low latitude surface box to the high latitude surface box before sinking into the deep box. p (mol C s^{-1}), Total particulate flux of organic and carbonate carbon. We assume that none is lost to sediments. We visualize the low latitude surface box as being 100 m thick and occupying 85% of the ocean's surface area. The high latitude surface box, which represents essentially the subpolar gyres, is 250 m thick and occupies the remaining 15% of the surface ocean.

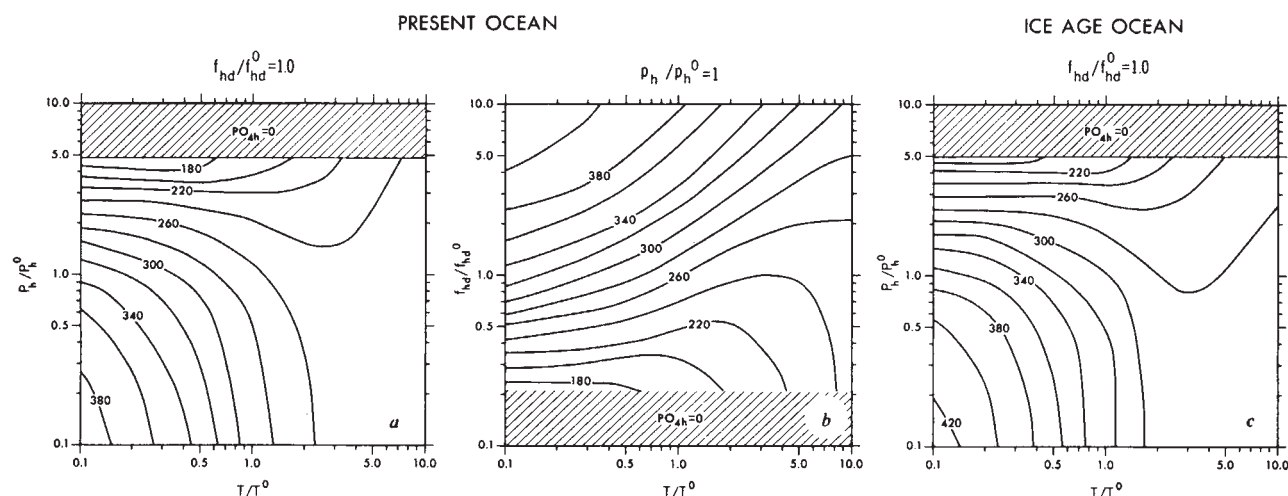


Fig. 3 Atmospheric P_{CO_2} (in p.p.m.) as predicted by our model for various values of the parameters f_{hd} , p_h and T . $f_{\text{hd}}^0 = 38.1 \times 10^6 \text{ m}^3 \text{ s}^{-1}$, $p_h^0 = 2.31 \times 10^6 \text{ mol C s}^{-1}$, and $T^0 = 24.5 \times 10^6 \text{ m}^3 \text{ s}^{-1}$. (See text for a description of the various scenarios.) The ice age ocean has an ocean volume that has been decreased by 3.5% and a total carbon and alkalinity content increased by 6%.

signal, and the precipitation of CaCO_3 which would have to accompany it, poses a dilemma. A time-dependent model is needed to study the problem in more detail.

Changes in the tilt of the Earth's axis, which would affect particularly the high latitude winter insolation, may actually drive the climate through the CO_2 response we have discussed here. The extremes within which the P_{CO_2} could conceivably vary with the present ocean volume, total carbon and alkalinity, and value of T , are ~ 190 p.p.m. to 300 p.p.m. If T were to approach zero the P_{CO_2} could vary between ~ 163 and 425 p.p.m. The climatic effect of P_{CO_2} changes within this range would be considerable. Manabe and Stouffer¹² as well as many others have shown that a halving or doubling of P_{CO_2} should lead to a decrease or increase of $\sim 2^\circ \text{C}$ in global mean temperature.

Changes in the oceanic carbon distribution by the processes represented by T , f_{hd} and p_h , may also have an impact on fossil fuel uptake. It is difficult to tell what the effect would be. A decrease in T and/or f_{hd} might be expected as high latitude surface waters warm up. A decrease in T and f_{hd} would affect the CO_2 uptake in opposite directions (see Fig. 3). We have not studied this problem, but suspect the effect of variations in T , f_{hd} and p_h on CO_2 uptake would be relatively small because of a tendency for the effects of T and f_{hd} to cancel each other out and because of the short time scales of the fossil fuel CO_2 transient relative to large-scale oceanic overturning.

Evidence of the types of changes we have suggested here for the ice ages should be available in sediment records. An increase in high latitude productivity should lead locally to fairly dramatic increases in sedimentation rates. The palaeo- PO_4 , which Boyle estimates by Cd measurements in foraminifera¹⁰, should be lower near deep water formation regions. Table 1 shows that the deep ocean oxygen levels would have decreased enormously in the extreme conditions of the ice age. One would expect anoxia to have been widespread at the depth of the oxygen minimum with possible effects on the sediments and trace metal distributions. We are presently seeking confirmation of our hypothesis in data of this sort, as well as working on the development of a three-dimensional ocean circulation model of the carbon cycle that will more realistically simulate the effect of high latitude productivity and thermohaline overturning rate on atmospheric P_{CO_2} .

We acknowledge support from ARL/NOAA grant NA83RAC00052, NSF grant OCE-8110155, and GFDL/NOAA grant 04-7-022-44017. We also appreciate the help of Sol Hellerman and Marty Jackson in data analysis, Johann Callan in typing the manuscript, and Phil Tunison in drafting

the figures. This paper is based on material first presented at a NATO Advanced Research Institute in July 1983. After this paper was accepted for publication we discovered that Oeschger *et al.*¹³ were the first to suggest the idea we explore here.

Received 16 November 1983; accepted 1 January 1984.

1. Neftel, A. H., Oeschger, H., Schwander, J., Stauffer, B. & Zumbunn, R. *Nature* **295**, 220–223 (1982).
2. Broecker, W. S. in *The Sea* Vol. 2 (ed. Hill, M. N.) 88–108 (Interscience, New York, 1963).
3. Sarmiento, J. L. *J. phys. Oceanogr.* **13**, 1269–1274 (1983).
4. Broecker, W. S. & Peng, T.-H. *Tracers in the Sea* (Eldigio, Palisades, 1982).
5. Broecker, W. S. *Geochim. cosmochim. Acta* **46**, 1689–1705 (1982).
6. Broecker, W. S. & Takahashi, T. *Ann. Glaciol.* (submitted).
7. Siegenthaler, U. *J. geophys. Res.* **88**, 3599–3608 (1983).
8. Redfield, A. C., Ketchum, B. H. & Richards, F. A. *The Sea* Vol. 2 (ed. Hill, M. N.) 26–75 (Interscience, New York, 1963).
9. Takahashi, T., Broecker, W. S., Bainbridge, A. E. & Weiss, R. F. *Tech. Rep. No. 1, CU-1-80* (Lamont-Doherty Geol. Observatory, Palisades, New York, 1980).
10. Boyle, E. A. & Keigwin, L. D. *Science* **218**, 784–787 (1982).
11. Streeter, S. S., Belanger, P. E., Kellogg, T. B. & Duplessey, J. C. *Quat. Res.* **18**, 72–90 (1982).
12. Manabe, S. & Stouffer, R. J. *J. geophys. Res.* **85**, 5529–5554 (1980).
13. Oeschger, H. *et al. Maurie Ewing Symp. 1982* Vol. 4 (American Geophysical Union, Washington DC, in the press).
14. Levitus, S. *Climatological Atlas of the World Ocean* (NOAA Professional Pap. 13, US Dept. of Commerce, Washington DC, 1982).

Rapid atmospheric CO_2 variations and ocean circulation

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Studies on air trapped in old polar ice^{1,2} have shown that during the last ice age, the atmospheric carbon dioxide concentration was probably significantly lower than during the Holocene—about 200 p.p.m. rather than 270 p.p.m. Also, Stauffer *et al.*³ recently showed by detailed analyses of Greenland ice cores, that during the ice age, between about 30,000 and 40,000 yr BP, the atmospheric CO_2 level probably varied between 200 and 260 p.p.m. These variations occurred parallel to climatic variations as indicated by $\delta^{18}\text{O}$ of the ice; astonishingly, the changes took place within rather short times, no more than a few centuries or even less. Here we examine the hypothesis⁴ that CO_2 variations arose from changes in ocean circulation that affected the distribution of chemical properties and thus of P_{CO_2} in the surface waters of the world ocean. Such changes can take place in a rather short time, in contrast to changes of whole ocean properties.

Table 1 Model parameters for the standard case

Properties	Warm surface	Cold surface	Whole ocean
Relative area	0.89	0.11	1
Depth (m)	200	200	3,700
Temperature (°C)	23	3	—
Salinity (‰)	35	35	35
ΣCO_2 ($\mu\text{mol kg}^{-1}$)	1,952	2,158	2,268
Alkalinity ($\mu\text{equiv. kg}^{-1}$)	2,315	2,360	2,389
Phosphate ($\mu\text{mol kg}^{-1}$)	0.30	1.43	2.15
Fluxes: 1 sverdrup (sv) = $10^6 \text{ m}^3 \text{ s}^{-1}$			
Water: $F_u = 15 \text{ sv}$; $F_{cd} = 44 \text{ sv}$; $F_{cw} = 10 \text{ sv}$			
Particles: $P_w = 0.64 \text{ mol C m}^{-2} \text{ yr}^{-1}$; $P_c = 0.93 \text{ mol C m}^{-2} \text{ yr}^{-1}$			
Gas exchange (for 300 p.p.m.): $F_{aw} = 19.5 \text{ mol C m}^{-2} \text{ yr}^{-1}$; $F_{ac} = 33 \text{ mol C m}^{-2} \text{ yr}^{-1}$			

Values in bold type were kept constant, the others were varied in sensitivity tests.

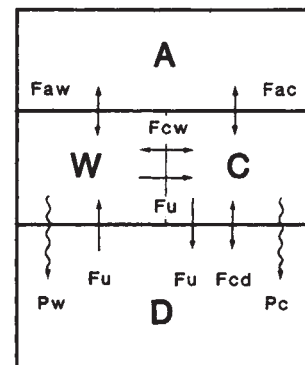
The ocean is the greatest of the rapidly exchanging global carbon reservoirs and therefore effectively controls the atmospheric concentration of carbon dioxide over time scales of less than 10^5 years. P_{CO_2} , the equilibrium partial pressure of CO_2 , of sea water is determined mainly by the temperature (via the solubility of CO_2 gas) and the carbonate chemistry of the water (ΣCO_2 and alkalinity), and to a lesser degree by salinity. During the glacial maximum at 18,000 yr BP, the average sea surface temperature was $\sim 2 \text{ K}$ lower⁵ and the average salinity of the ocean $\sim 1\%$ higher than now. Temperature and salinity changes together would have caused a net decrease of the atmospheric CO_2 concentration of 10–20 p.p.m., compared with Holocene conditions⁷. This is much less than the observed difference of 50–70 p.p.m.; this difference must therefore have been caused by chemical changes in surface waters.

ΣCO_2 in average surface seawater is 10–20% lower than in deep-sea water, because biological activity provides a continuous carbon flux in the form of organic and carbonate particles from the surface to deeper layers. It is the interplay of biological activity and transport by water circulation which mainly regulates ΣCO_2 , and therefore P_{CO_2} , in the surface waters. In large areas of the ocean, biological productivity is limited by the nutrients phosphate and nitrate, which are depleted there to near zero. In these areas the biological pump is closely linked to water circulation: a change in the vertical circulation rate, and therefore in supply of nutrients from depth, involves a proportional change in biological activity, so that the chemical concentrations in surface waters remain unaffected.

Broecker^{6,7} suggested that during glacial times, the mean oceanic concentration of nutrients was considerably higher than in the Holocene, thus permitting, for the same water circulation pattern, a higher biological productivity and therefore a larger surface deficit of ΣCO_2 , compared with deep-sea water which would lead to an overall lower ΣCO_2 and P_{CO_2} in surface water. The mechanism leading to the nutrient increase, as suggested by Broecker, was erosion of nutrient-rich organic sediments on the continental shelves, deposited there during the last interglacial high sea level period. This hypothesis could explain glacial–postglacial changes of atmospheric CO_2 as well as of marine $\delta^{13}\text{C}$. However, deposition and erosion of shelf sediments are slow processes and bound to major changes in sea level. Therefore, they cannot have been responsible for the recently detected rapid CO_2 variations during the Ice Age³.

Significant surface concentrations of phosphates and nitrates are found in some oceanic regions where vertical mixing is rather fast, especially in the Antarctic Ocean where the phosphate concentration is about 70% of its deep-water value of $2.2 \mu\text{mol kg}^{-1}$; in warm surface water, it is typically $\sim 10\%$. Thus, the biological productivity in the Antarctic Ocean is not limited by availability of nutrients, therefore changes in either productivity or circulation may lead to changes in the carbonate chemistry and hence in the P_{CO_2} of Antarctic surface water. In the North Atlantic Ocean, where deep water formation also occurs, surface nutrient concentrations are generally low.

Fig. 1 Four-box model consisting of warm surface ocean (W), cold surface ocean (C), deep-sea reservoir (D) and atmosphere (A). F_{cd} , F_{cw} , F_u (= upwelling flux) are water fluxes; F_{aw} , F_{ac} , air–sea gas exchange fluxes; P_w , P_c , particle fluxes.



To estimate the effects of various changes in the ocean, we have used a simple 4-box model of ocean and atmosphere (Fig. 1). Box C represents the Antarctic Ocean south of 50°S . Temperatures and chemical concentrations (all normalized to a salinity of 35‰; see Table 1) have been chosen essentially in agreement with averaged GEOSECS results⁸. Phosphate is considered to be the limiting nutrient; taking nitrate instead would not affect our results. ΣCO_2 , alkalinity and phosphate are transferred by means of water fluxes between the three boxes and by particle fluxes from surface to depth.

The ratios of carbon, alkalinity and phosphorus carried by biogenic particles are fixed as $\Delta\Sigma\text{CO}_2 : \Delta\text{A} : \Delta\text{PO}_4 = 155 : 35 : 1$. These generalized Redfield ratios were obtained by assuming (1) a ratio of 130:1 for $\Delta\Sigma\text{CO}_2 : \Delta\text{PO}_4$ in organic matter⁹; (2) an average ratio of inorganic (CaCO_3) to organic carbon in particles of $\sim 0.2:1$; (3) carbonate alkalinity accounts for 70% of the change in titration alkalinity, which is affected also by nitrate carried by organic particles. The water exchange fluxes to the deep sea for Holocene conditions can roughly be estimated from mean ^{14}C concentrations of 0.84 for the deep sea and ~ 0.91 for the cold surface (the pre-industrial atmospheric value is 1). This yields a mean ^{14}C age of deep-sea water of ~ 700 yr with respect to the cold surface from which it originates. Thus, the flux between the cold surface and the deep sea approximately corresponds to a transfer velocity of $3,500 \text{ m per } 700 \text{ yr} = 5 \text{ m per yr}$, referring to the whole ocean area, equivalent to about $60 \times 10^6 \text{ m}^3 \text{ s}^{-1}$ (60 sverdrup). We have chosen a standard case believed to represent Holocene conditions (see Table 1). The particle fluxes are computed such that the imposed chemical properties are reproduced; their values of 0.93 and $0.64 \text{ mol C m}^{-2} \text{ yr}^{-1}$ from cold and warm surface are comparable with the values estimated from observation¹⁰, that is, $0.34\text{--}0.83 \text{ mol C m}^{-2} \text{ yr}^{-1}$. The surface boxes exchange CO_2 via the atmosphere by gas transfer. The gas transfer fluxes are proportional to P_{CO_2} ; for $P_{\text{CO}_2} = 300$ p.p.m. they are 33 and $19.5 \text{ mol C m}^{-2} \text{ yr}^{-1}$ for cold and warm surface water, respectively. The difference is due to the higher solubility and higher transfer velocities of CO_2 in cold water, as derived from radon measurements in the Antarctic Ocean¹¹. P_{CO_2} values in surface water are calculated using a procedure described by Bacastow¹². For the standard case an atmospheric concentration of 282 p.p.m. is obtained.

By using the model we can test the effect of different processes on atmospheric P_{CO_2} and its isotopic composition. For these tests, the phosphate concentration in warm surface water is considered constant, arguing that PO_4 is almost completely consumed by organisms. Also, the particle flux from the cold surface and the total oceanic inventories of all properties are assumed to be constant. First, the exchange C–D is reduced to 50% of its standard value (Table 2). This causes a decrease of ΣCO_2 and of P_{CO_2} , because the biological particle flux, while absolutely constant, becomes relatively more important. The atmospheric CO_2 concentration decreases by 52 p.p.m., a difference comparable to the CO_2 changes found in ice cores. An interesting result is that P_{CO_2} in W changes drastically from 283 to 233 p.p.m., although the circulation change does not directly influence W. This is a result of a net carbon transport from W to C via the atmosphere¹³. The importance effect of atmospheric

Table 2 Sensitivity tests; properties in the reservoirs for atmosphere (A), and warm (W) and cold (C) surface ocean

		Particle flux (mol C m ⁻² yr ⁻¹)	PO ₄ (μmol kg ⁻¹)	Standard gas exchange			Gas exchange → O		
				ΣCO ₂ (μmol kg ⁻¹)	P _{CO₂} (p.p.m.)	δ ¹³ C (‰)	ΣCO ₂ (μmol kg ⁻¹)	P _{CO₂} (p.p.m.)	δ ¹³ C (‰)
Standard case (F _u = 15 sv, F _{cd} = 44 sv)	W	0.64	0.30	1,952	283	1.6	1,981	323	2.6
	C	0.93	1.43	2,158	276	1.4	2,156	274	1.3
	A	—	—	—	282	-6.9	—	315	-6.0
Reduced exchange C-D (F _u = 15 sv, F _{cd} = 22 sv)	W	0.58	0.30	1,910	233	2.0	1,981	323	2.6
	C	0.93	1.05	2,101	215	1.8	2,097	211	1.7
	A	—	—	—	230	-6.4	—	304	-5.8
Stronger upwelling (F _u = 30 sv, F _{cd} = 44 sv)	W	1.08	0.30	1,934	260	2.1	1,981	323	2.6
	C	0.93	1.23	2,127	241	1.5	2,125	238	1.5
	A	—	—	—	257	-6.4	—	308	-5.9

CO₂ transport is illustrated by reducing the air-sea exchange fluxes to nearly zero (10⁻⁴ of their standard values): now the same reduction of the water exchange C-D causes an atmospheric drop of only 11 p.p.m. and does not affect P_{CO₂} in W at all (see Table 2). ΣCO₂ and P_{CO₂} in C are less affected by the gas exchange than in W, because C is strongly coupled to the deep sea by a large water-exchange flux.

The model includes a deep-water formation—upwelling circulation, F_u. If F_u is increased from 15 to 30 sverdrup, the atmospheric CO₂ decreases by 25 p.p.m. Thus an increase in low-latitude upwelling, accompanied by higher biological productivity in low latitudes, leads to a smaller atmospheric CO₂ concentration. The reason is found again in the cold surface water, which is diluted by low-latitude water depleted in ΣCO₂. The enhanced upwelling of ΣCO₂ (and nutrients) to the warm surface is automatically compensated by a larger particle flux, since a constant PO₄ concentration is assumed in W.

A change of the surface exchange flux, F_{cw} has an effect comparable to that of varying the upwelling circulation. An increase of F_{cw} from 10 to 20 sverdrup results in an atmospheric concentration decrease of 19 p.p.m.

Data from the eastern equatorial Pacific indicate that the δ¹³C difference between warm surface and deep water during the glacial maximum was about 0.5‰ greater than during the Holocene²⁵. Our 4-box model yields changes of this magnitude for both scenarios, reduced C-D exchange and enhanced upwelling circulation. The sediment data²⁵, however, also point to significant long-term δ¹³C changes in average deep water, which cannot be explained by circulation changes (δ¹³C in the deep sea is constant within 0.03‰ in the different model scenarios considered here). These changes must be due to carbon shifts between the ocean and organic carbon pools of either terrestrial biomass¹⁵ or shelf sediments^{6,7}. Information on δ¹³C changes in atmospheric CO₂ should soon be available from analyses of ice cores performed in this laboratory.

These examples demonstrate that changes of the oceanic circulation, especially those directly affecting the Antarctic Ocean, may potentially cause changes of atmospheric CO₂. Is there any evidence for such circulation changes during the glacial? Sediment studies in the Vema channel¹⁶ indicate a stronger flow of Antarctic Bottom Water into the Atlantic Ocean at 18,000 BP. This may indicate a stronger upwelling—deep water formation circulation (see Table 2). The wind-induced circulation in the tropics was stronger at 18,000 BP in the Atlantic¹⁴ and probably also in the eastern equatorial Pacific Ocean where productivity was increased¹⁷; it is not clear if this involved a stronger flux from low to high latitudes or whether it was restricted to shallow recirculation in low latitudes. A number of deep-sea sediment studies in the North Atlantic concerning temperature¹⁸, δ¹³C (ref. 19), oxygen²⁰ and cadmium concentrations²¹ have demonstrated that the formation of North Atlantic deep water was significantly reduced during the last glacial. Ruddiman and MacIntyre²² have shown that the surface circulation—and probably also deep water formation—in the North Atlantic Ocean underwent rapid and drastic changes during late glacial times, ~13,000 to 10,000 BP. However, these changes probably did not exert a major direct

effect on atmospheric CO₂, because nutrient concentrations are low in North Atlantic surface water. We know of no similar studies for the Antarctic Ocean. Thus, there is ample evidence for dramatic and rapid changes of the circulation in the North Atlantic, but it is not clear how this affected the Antarctic Ocean which is the most critical region for atmospheric CO₂ variations.

Thus, we propose that changes of the surface ocean circulation were responsible for rapid atmospheric CO₂ variations during and at the end of the last Ice Age. The variations predicted by the model are fast since the residence times of carbon in the surface reservoirs are of the order of a century only. The simple 4-box model used here cannot be expected to yield final or quantitatively reliable results, but it indicates that the Antarctic Ocean may have a major effect on the level of atmospheric CO₂.

Independently of this study, Knox and McElroy²³ and Sarmiento and Toggweiler²⁴ discuss the idea that the Antarctic Ocean, in which ΣCO₂ is not fixed by the availability of nutrients, may be responsible for atmospheric CO₂ variations. These authors assume that changes in light conditions led to changing biological productivity and thus affected P_{CO₂}. We rather think that ocean circulation changes were the essential cause. Besides this, there are differences between the results of these authors and of ours, due to differing assumptions on fluxes, concentrations and model geometry.

This work was supported by the Swiss NSF. This paper is based on ideas formulated in ref. 4 and presented at the IUGG Symposium on the Ocean and the CO₂ Climate Response, Hamburg, August 1983. We thank H. Oeschger for stimulating discussions.

Received 9 January; accepted 1 February 1984.

1. Neftel, A., Oeschger, H., Schwander, J., Stauffer, B. & Zumbund, R. *Nature* **295**, 220–223 (1982).
2. Delmas, R. J., Ascencio, J. M. & Legrand, M. *Nature* **284**, 155–159 (1980).
3. Stauffer, B., Hofer, H., Oeschger, H., Schwander, J. & Siegenthaler, U. *Ann. Glaciol.* **5**, (in the press).
4. Oeschger, H. *et al. Maurice Ewing Symp. 1982 Vol. 4* (American Geophysical Union, in the press).
5. CLIMAP Science **191**, 1131–1137 (1976).
6. Broecker, W. S. in *Climatic Variations and Variability: Facts and Theories* (ed. Berger A.) 111–121 (Reidel, Dordrecht, 1981).
7. Broecker, W. S. *Geochim. cosmochim. Acta* **46**, 1689–1705 (1982).
8. Takahashi, T., Broecker, W. S. & Bainbridge, A. E. in *Carbon Cycle Modelling, SCOPE 16* (ed. Bolin, B.) 159–200, 271–286 (Wiley, New York, 1981).
9. Broecker, W. S., Takahashi, T. & Langer, J. *mar. Res.* (in the press).
10. Mopper, K. & Degens, E. T. in *The Global Carbon Cycle, SCOPE 13* (eds Bolin, B., Degens, E. T., Kempe, S. & Kerner, P.) 293–316 (Wiley, New York 1979).
11. Peng, T.-H., Broecker, W. S., Mathieu, G. G. & Li, Y.-H. *J. geophys. Res.* **84**, 2471–2496 (1979).
12. Bacastow, R. B. in *Carbon Cycle Modelling, SCOPE 16* (ed. Bolin, B.) 95–101 (Wiley, New York, 1981).
13. Broecker, W. S. & Takahashi, T. *Ann. Glaciol.* **5**, (in the press).
14. Gardner, J. V. & Hays, J. D. *Mem. geol. Soc. Am.* **145**, 221–246 (1976).
15. Shackleton, N. J. in *The Fate of Fossil CO₂ in the Oceans* (eds Andersen, N. R. & Malahoff, A.) 401–427 (Plenum, New York, 1977).
16. Ledbetter, M. T. & Johnson, D. A. *Science* **194**, 837–839 (1976).
17. Pedersen, T. F. *Geology* **11**, 16–19 (1983).
18. Duplessy, J. C., Chenouard, L. & Vila, F. *Science* **188**, 1208–1209 (1975).
19. Shackleton, N. J., Imbrie, J. & Hall, M. A. *Earth planet. Sci. Lett.* **65**, 233–244 (1983).
20. Streeter, S. S. & Shackleton, N. J. *Science* **203**, 168–171 (1979).
21. Boyle, E. A. & Keigwin, L. D. *Science* **218**, 784–787 (1982).
22. Ruddiman, W. F. & MacIntyre, A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **35**, 145–214 (1981).
23. Knox, F. & McElroy, M. B. *J. geophys. Res.* (in the press).
24. Sarmiento, J. L. & Toggweiler, J. R. *Nature* (in the press).
25. Shackleton, N. J., Hall, M. A., Line, J. & Shui, C. *Nature* **306**, 319–322 (1983).

Structural evolution of Lake Malaŵi, Africa

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Over 2,000 km of seismic reflection profiles have been collected in Lake Malaŵi (Nyasa), the southernmost of the East African Rift Lakes. These studies, reported here, reveal a severely faulted lake floor bounded by rift structures of Pliocene or greater age. Faults that penetrate the uppermost sedimentary units and numerous earthquakes¹ attest to continuing activity in the rift. Inter-basinal differences in fault spacing, orientation and sedimentary thicknesses have led to a subdivision of Lake Malaŵi into four discrete structural provinces. These provinces only now are linked by faults attributed to the most recent episodes of extension. An evolutionary sequence of: (1) initial block faulting and subsidence, (2) fragmentation of fault blocks, (3) uplift and (4) renewed subsidence and rotation of fault blocks is proposed for the central part of the Malaŵi Rift.

Single-channel reflection surveys were conducted as part of a programme to evaluate sites for a proposed deep-drilling project (CEGAL) in the East African Rift Lakes². Profiles were obtained using an airgun sound source (40 cubic inches) occasionally supplemented by an 800 J sparker, and radar navigation provided the principal means of trackline control. A single 13-m-long piston core was collected from the southern part of the lake (Fig. 1).

With a length of 560 km and an average width of 50 km, Lake Malaŵi fills the central valley of the Malaŵi Rift (Fig. 1). The lake floor reaches a maximum depth of 700 m, or 225 m below sea level³. Structural patterns⁴ and geophysical data⁵ indicate that the Malaŵi Rift is tectonically connected to the western branch of the East African Rift system. Agreement on the age of initiation of the Malaŵi Rift has been hampered by the paucity of reliable field data and rock dating. Earlier researchers believed rifting began in the Jurassic-Cretaceous^{6,7}, but recent workers have suggested a much younger Pliocene age⁴. Faunas from the earliest known lacustrine sediments exposed onshore, the Chiwondo Beds, have been dated at 2.5–5 Myr⁸ and are separated from the overlying Chitimwe Beds by an erosional event of unknown duration⁹.

The surface geology to the west of Lake Malaŵi has been reported previously¹⁰, but little information is available as to the age of faulting on the eastern shores (Tanzania and Mozambique). The results of a recent research programme along the Tanzanian shores of Lake Malaŵi indicate that the large vertical offset along the Livingstone Fault (Fig. 1) is accompanied by an even greater amount of horizontal displacement (J. LeFournier, personal communication). Before our seismic survey, knowledge of the lake floor had been derived from a reconnaissance heat flow survey¹¹, a few echograms from the south-west arm of the lake³ and a theoretical study of lake bed topography¹². Sediment distribution was poorly known, but sand, mud, diatomite and iron precipitates were found in the uppermost layers¹³.

Set within the roughly north-south boundary fault system shown in Fig. 1, the Malaŵi Rift transects Precambrian metamorphic basement and north-east-trending Mesozoic (Karoo) fault troughs. Some reactivation of pre-existing zones of weakness has been described onshore⁴, and our seismic records show that submerged segments of the Maniamba and Ruhuhu Karroo fault troughs have been reactivated. The segmented character of the boundary fault system and inter-basinal differences in

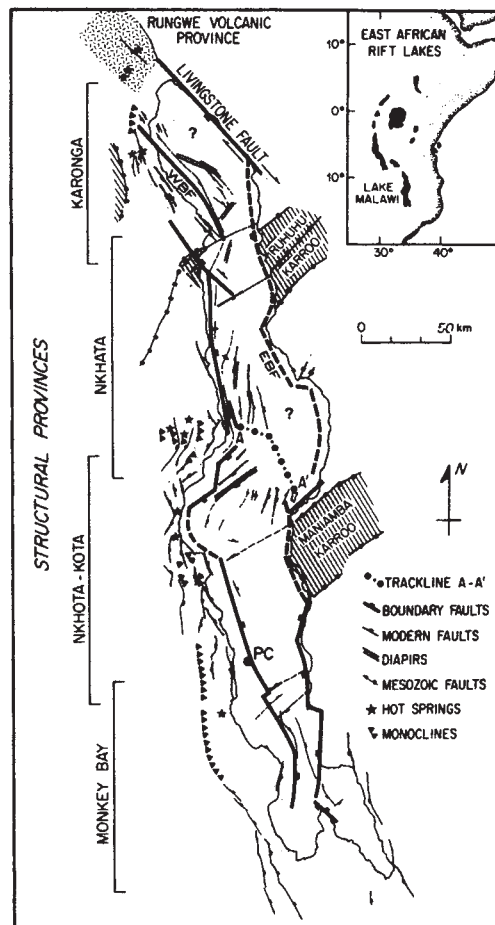


Fig. 1 Tectonic map of Lake Malaŵi region (lakeshore shaded for clarity) showing the principal structural provinces that comprise the lake basin. Faults with large offsets roughly defining the sedimentary basins are designated as Eastern Boundary Fault (EBF) and Western Boundary Fault (WBF). Note that these occur within the monoclines bounding the Malaŵi Rift Valley. PC denotes location of the 13-m piston core. Symbols: diagonal line pattern, Mesozoic sediment troughs; random dashes, Rungwe volcanics.

seismic stratigraphy lead to a division of the rift into four discrete structural provinces. Each province has a distinct fault pattern, and few faults extend between provinces; therefore, principal stress orientations are inferred to shift at province boundaries (Fig. 1). The provinces are typically 60 km long and 50 km wide, with the cross-sectional forms of half-graben. The main graben are composed of many closely spaced, usually tilted, fault blocks. Faults penetrate to all levels of the sedimentary column and diapiric features occur along some faults (Figs 2, 3).

The four provinces (Fig. 1) reflect different stages of rift evolution, with the youngest, the Monkey Bay Province, showing the least amount of subsidence and fragmentation of major fault blocks. Maximum subsidence has occurred along the Western Boundary Fault in the Nkhata Province where sediment thicknesses are also greatest. A structural interpretation of the southern Nkhata Province is shown in the block diagram of Fig. 2. Up to 2.0 km of subsidence is observed in the seismic records, but the actual amount could be much greater as basement was often obscured by the bottom multiple (Fig. 3). Steeply dipping synthetic and antithetic normal faults trend roughly north-south within this province. Shallow earthquakes of $m_b \geq 3$ correlate well with mapped structures and appear to be related to movements along the Western Boundary Fault and a mid-lake basement high (Fig. 2).

The southern part of the Nkhata Province contains many north-east-trending faults (Fig. 2) with little or no vertical offset. These are interpreted as strike-slip faults that link this part of

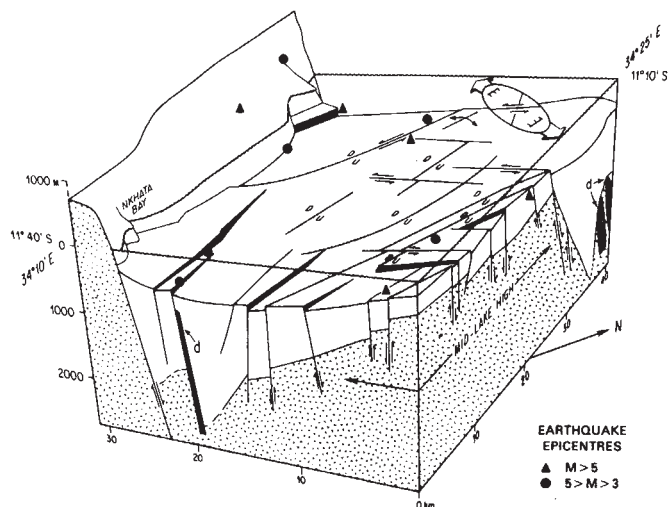


Fig. 2 Schematic block diagram depicting the major structural elements of the southern Nkhata Province. (Interpretation based on seismic reflection profiles shown in inset to Fig. 3.) The strain ellipse superimposed on the lake surface indicates approximate orientation of principal strain axes inferred from fault geometries. Faults with a north-east trend correspond to planes of high shear strain, and north-south-trending faults lie along planes normal to the principal extension direction. Epicentres of earthquakes of $m_b \geq 3$ (1966–77) are shown in their approximate locations. Symbols: random Vs, crystalline basement; d, diapirs; blank, lake sediments.

the Nkhata Province to the Nkhota-kota Province near the bend in the lake. A zone of transcurrent movement mapped onshore in this area¹⁴ supports a strike-slip interpretation. In Lake Malaŵi the propensity towards half-graben morphologies could be related to oblique extension across the lake or between individual segments, as basin asymmetry alternates between provinces. Where rift segments are offset along strike this oblique extension would introduce a strong component of horizontal shear into the regional tensional regime. A similar alternating pattern of basinal asymmetry and transcurrent faults has been observed in the Rio Grande Rift¹⁵.

Diapiric features observed along some faults in this area (Figs 2, 3) could be related to the period of uplift which created or enhanced a mid-lake high. Although no rift-related igneous activity has been described onshore in the Nkhata region, the Rungwe volcanic zone to the north¹⁶ (Fig. 1) has been active since the Pliocene. These diapirs could be Rungwe-related igneous intrusive bodies or the lake bed equivalent of hydrothermal springs that occur along the Western Boundary Fault (Fig. 1).

Many of the previously described features and relations are observed in a seismic line across the southern part of the Nkhata Province near the bend in Lake Malaŵi (Fig. 3). The nearly cross-sectional seismic section shown in Fig. 3 serves as the basis for the evolutionary sequence depicted in Fig. 4. To remove non-diastrophic effects from the lake bottom morphology, reconstructions were made to acoustic horizons that are presumed to be time-stratigraphic. Information from nearby seismic lines was also used in the reconstructions. The Western Boundary Fault serves as a fixed point of reference for all four stages to illustrate the relative amount of extension that has occurred along this transect.

Little is known of the pre-rift terrains of central Africa. Therefore, stage 1 depicts depositional pulses in an already faulted basin (horizon A). Reconstruction to the top of acoustic (presumably crystalline) basement indicates that the graben developed on the western margin of a broad dome. The north-south elongate rift apparently interrupted a west-to-east drainage pattern, capturing detritus derived predominantly from the west. The discontinuous, fuzzy reflectors of the lowermost acoustic unit are interpreted as the earliest Malaŵi Rift sediments,

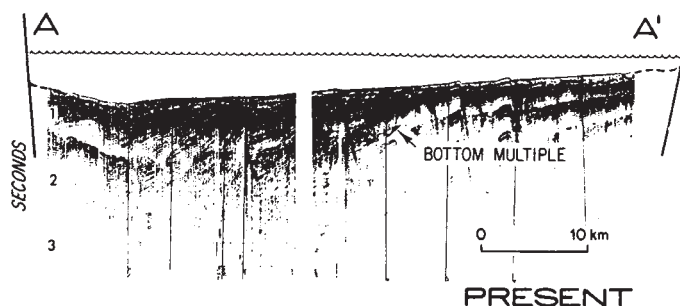


Fig. 3 Seismic reflection profile A-A' (see Fig. 1 for location) referred to in Fig. 4. Vertical scale in seconds two-way travel time. Horizontal extent ~70 km.

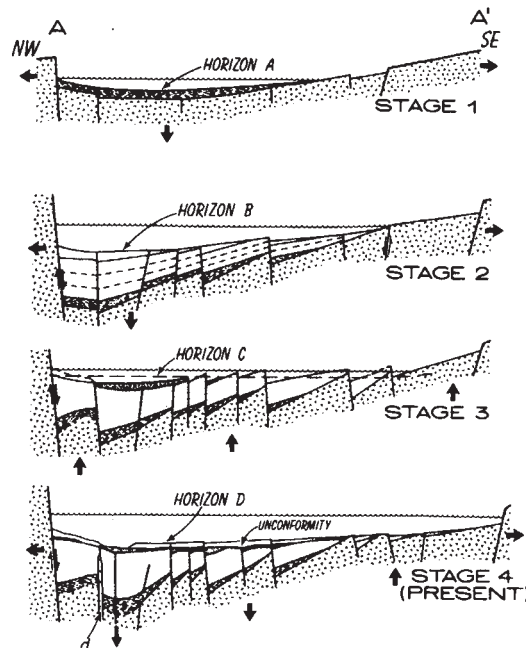


Fig. 4 Four-stage evolutionary sequence envisioned for trackline A-A' (see Fig. 3). Horizons A–D refer to seismic horizons used as datum in the reconstructions. Arrows denote relative movements within the lake. Symbols as in Fig. 2.

probably sands shed from the uplifted rift flanks. These sediments could be the equivalent of the shallow lake and alluvial plain deposits of the exposed Chiwondo Beds^{9,10}. The wedge-shaped geometry of this basal unit suggests that a few faults in the central valley were active during the initial infill stages.

The period between stages 1 and 2 was one of extension and rapid subsidence as rift-flank drainage patterns became more extensive. At the time corresponding to stage 2, the boundary faults were well established and the rift had achieved most of its present width. Subsidence along the western fault proceeded much more rapidly than its eastern counterpart, and this asymmetry was enhanced by clastic input largely from the west. The parallel-banded reflectors in this middle unit are indicative of deep-water deposition of muds (via turbidity currents?) and biogenic oozes, with lake conditions presumed to be similar to those prevailing today. The geometry of acoustic laminations shows deposition on an uneven lake floor concomitant with rotation of fault blocks.

Stage 3 is a reconstruction to the top of a strongly reflective, nearly horizontal layer (horizon C) that appears to truncate the uplifted corners of rotated fault blocks. As deposition on continuously tilting fault blocks can account for only part of the reflector inclinations at this level, an erosional unconformity is proposed. Further evidence for a period of relative uplift is seen in the central lake area where a structural basement high developed (Fig. 2). The intertonguing bed pattern in the adjacent

central graben indicates sediments derived not only from the west but from a shallow or subaerially exposed mid-lake high. An apparently short-lived period of uplift in segments of Lake Malaŵi may be analogous to the mid-Jurassic doming episode observed in the central North Sea area¹⁷, and to doming in the central basin of Lake Tanganyika². Two major erosional events described for central Africa¹⁸ could be related to drier periods and lowstands of the lake, but too little is known of the timing, duration or extent of these events in the region surrounding Lake Malaŵi to make any well-constrained correlations at this time.

Stage 4 is an interpretation of the present-day structural and stratigraphic relations in the Nkhata Basin of Lake Malaŵi. Extension, rotation and continued fragmentation of fault blocks dominated the latest rift episode. Substantial movements along the border fault system led to general subsidence, while the lake refilled to its present level. The unconformity surface in the Nkhata Province was overlain by a 60-m thick, acoustically 'transparent' unit. Core studies of the upper 13 m show this to be a diatomite with minor clays and organic debris. In the extensional periods between stages 1 and 2 the border faults became more diffuse. The more rapid subsidence between stages 3 and 4 was largely restricted to a 20-km wide central graben and was achieved by vertical and rotational movements along a few major faults. A similar narrowing of the zone of maximum subsidence has also been observed in the South Kenya Rift¹⁹.

An extrapolation of the chronological history of the Nkhata Province to the remaining lake provinces indicates that Lake Malaŵi has grown through the coalescence of structural basins during successive episodes of regional extension, uplift and subsidence. Half-graben morphologies and fault patterns with strike-slip geometries (north-east-trending faults) indicate that the Malaŵi Rift is opening obliquely to the axis of the rift (north-south). This is most apparent in the region surrounding the bend in the lake, which could evolve into a transform zone if the Malaŵi Rift becomes a true spreading system.

The areal dimensions of the four structural provinces (60 km × 50 km) are remarkably similar within Lake Malaŵi, the East African Rift System and many other continental rifts. Fault patterns within the rift are influenced by pre-existing structural grains, but the dimensions of basins in the Malawi Rift remain fairly constant. Rosendahl and Livingstone² have suggested that the recurrence of these dimensions reflects a fundamental response of the lithosphere to the process of rifting that is only secondarily affected by pre-existing structural grains. Further studies in the rift lakes of East Africa, where it may be possible to observe continental separation in its juvenile stages, are needed to define the vertical and horizontal extent of basement structures within the extensional basins, and to relate the observed repeatability of dimensions to crustal, lithospheric or asthenospheric properties.

This paper was made possible by the Government of Malaŵi and the Malaŵi Geological Survey Department. We thank J. Turner, J. Barrett; the scientific crew of R. Dunseath, J. Nelson, M. Patterson, F. Spy-Anderson, K. Haberyan and C. Stager; M. Connor for the artwork; J. Karson for critical comments; and E. Uchupi for reviewing the paper. The work was supported by NSF grant BNS 7926762, Société Nationale Elf Aquitaine (Production), and Mobil Oil Corporation.

Received 12 September 1983; accepted 5 January 1984.

1. Fairhead, J. *20th a. Rep. res. Inst. Afr. Geol. Univ. Leeds*, 42–46 (Leeds Univ., 1976).
2. Rosendahl, B. & Livingstone, D. *Episodes* 1983, 14–19 (1983).
3. Eccles, D. *Limnol. Oceanogr.* 19, 730–742 (1974).
4. Crossley, R. & Crow, M. *Geodyn. Evol. Afro-Arab. Rift Syst.*, 77–87 (Accad. Nazion. dei Lincei, 1980).
5. Girdler, R. & Sowerbutts, W. J. *Geomag. Geoelect.* 22, 153–162 (1970).
6. Dixey, F. Q. *Jl geol. Soc. Lond.* 83, 75–108 (1929).
7. Bloomfield, K. & Garson, M. *Bull. geol. Surv. Malaŵi* 17 (1965).
8. Kaufulu, Z., Vrba, E. & White, T. *Ann. Transv. Mus.* 33, 1–8 (1980).
9. Crossley, R. *Paleoecol. Afr.* 15, 139–144 (1982).
10. Carter, G. & Bennett, J. *Bull. geol. Surv. Malaŵi* 6 (1973).
11. Von Herzen, R. & Vacquier, V. *Geophys. Res.* 72, 4221–4226 (1967).
12. Yairi, K. *2nd Prelim. Rep. Afr. Stud. Nagoya Univ.*, 51–69 (Nagoya Univ., 1977).
13. Muller, G. & Forstner, U. *Miner. Deposita* 8, 278–290 (1973).
14. Bloomfield, K. *Nature* 211, 612–614 (1966).

15. Cape, C., McGeary, S. & Thompson, B. *Geol. Soc. Am.* 94, 3–14 (1983).
16. Harkin, D. *Mem. Geol. Tanganyika* 2 (1960).
17. Ziegler, P. *Nature* 304, 561 (1983).
18. Lister, L. *Rec. geol. Surv. Malaŵi* 7, 15–28 (1967).
19. Baker, B., Crossley, R. & Gole, G. *Petrology and Geochemistry of Continental Rifts* (eds Neumann, E. & Ramburg, I.) 62–78 (Reidel, Dordrecht, 1978).

No geochemical evidence for an asteroidal impact at late Devonian mass extinction horizon

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One way to test the proposed causal relationship between mass biotic extinction and asteroidal impact is to search for the geochemical signature of such an event at additional mass extinction horizons other than the Cretaceous–Tertiary (K–T) boundary. We report here the results of an extensive search for an iridium (Ir) anomaly across the late Devonian Frasnian–Famennian (F–F) extinction horizon, ±367 Myr BP. Over a decade ago, McLaren¹ proposed that this extinction was caused by an astrophysical catastrophe. We have examined three sedimentary sequences in New York State (the standard reference section for eastern North America) and one in Belgium (the type locality) which cross the F–F boundary. No Ir anomaly was found in any of the analysed stratigraphic sections. The highest values of Ir detected ranged from only 0.2% to 2% of those reported for the marine and terrestrial Ir analyses at the K–T boundary. Further, Devonian pyrite-rich sediments similar to those at the K–T boundary do not contain high Ir concentrations.

The proposal by Alvarez *et al.*² that an impacting asteroid was the cause of major extinctions at the end of the Cretaceous has given rise to a topical conference (held in Snowbird, Utah in 1981) and lively discussion in the literature. Testing the hypothesis that asteroid impact can stress biological systems sufficiently to cause worldwide extinctions is difficult and, so far, heavily model-dependent. One test of the hypothesis is to search for geochemical anomalies in other, well-established worldwide extinction boundaries in the Phanerozoic³. We chose the Frasnian–Famennian (F–F) (late Devonian) boundary for analysis, as it also corresponds to a global extinction event⁴.

Geological sections having continuous marine sedimentation across the F–F boundary are present in New York State (the Devonian standard reference section for eastern North America), Belgium (the type section of the Frasnian and Famennian), Alberta, Western Australia, Poland and south China. The fact that a major drop in species diversity, and a marked change in faunal composition, occurs during the late Devonian epoch was recognized by palaeontologists and stratigraphers in New York well over a century ago. By way of illustration, worldwide stromatoporoid-coral reef ecosystems were decimated, along with an estimated 86% of all brachiopod genera and 90% of the phytoplankton⁵. This faunal break was utilized to divide the New York Upper Devonian strata into two 'series', now designated the Senecan and the Chataquan⁶. The extinction horizon is easily recognizable through the Appalachian Basin on the basis of brachiopods^{7,8}. Stratigraphically, this faunal break correlates with the basal Dunkirk Shale horizon in New York. Strata below the Dunkirk Shale contain an abundant and characteristic assemblage of Frasnian shellfish; these are absent and only a very depauperate fauna is preserved in strata immediately overlying the basal Dunkirk^{8,9}.

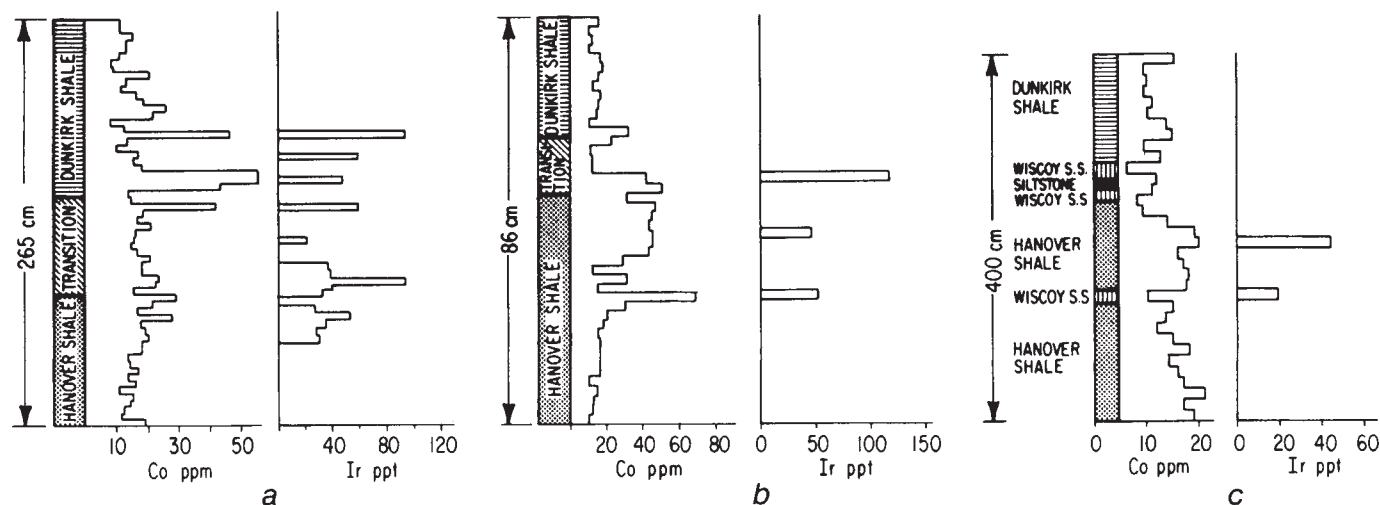


Fig. 1 Variations of Co and Ir with depth for the Frasnian-Famennian boundary sections at Walnut Creek (a), Dunkirk Beach (b), and Mills Mills (c), New York State.

There is some disagreement as to the formal placement of the F-F boundary with respect to intercontinental ammonoid and conodont zonation¹⁰⁻¹⁵. In Europe, the F-F break has traditionally been defined in terms of ammonoid zonation, the stage boundary being placed at the boundary between the *Manticoceras* Stufe (Frasnian) and the *Cheiloceras* Stufe (Famennian). Uncertainty exists in the placement of the boundary relative to the more recently developed conodont zonation; the F-F boundary may either correspond to the *Palmatolepis gigas*-Lower *triangularis*, Lower-Middle *triangularis*, or Middle-Upper *triangularis* horizons¹⁰⁻¹⁵. It is generally agreed, however, that conodonts of the Upper *triangularis* subzone are Famennian in age. Conodonts of this subzonal interval occur in the Dunkirk Shale in New York, while ammonoids of the *Manticoceras* Stufe (Frasnian) occur in the underlying Hanover Shale⁶. Thus, the F-F boundary in Europe appears to correlate with the Senecan-Chataquan boundary (Hanover-Dunkirk) in New York.

Three stratigraphic sections were chosen in northwestern New York which cross the Hanover-Dunkirk interval: Dunkirk Beach (the type locality of the Dunkirk Shale), Walnut Creek Gorge, and Mills Mills (see refs 16-18). The Dunkirk Beach and Walnut Creek Gorge field areas were located in deep water (150-200 m) basin axis regions of the Appalachian epicontinental sea during the late Devonian. Sedimentation rates¹⁹ were in the range 5-10 mm kyr⁻¹. Sediments at the Mills Mills section were deposited in shallower water delta slope regions (50-100 m), sedimentation rates 10-20 mm kyr⁻¹. Lithologies range from grey shales and siltstones in shallower water regions, grading to black (anoxic) shales with pyrite nodules in deeper water regions. A total thickness of 200 cm was sampled at Dunkirk Beach and 265 cm at Walnut Creek Gorge. At Mills Mills, a total thickness of 400 cm was sampled.

One stratigraphic section was sampled at Sinsin, in southern Belgium²⁰, which is the type locality of the Frasnian and Famennian Stages. Sediments here were also deposited in an epicontinental sea setting (the Dinant Basin), and are chiefly calcareous siltstones and shales. A total thickness of 400 cm was sampled, 20 cm above the F-F boundary and 380 cm downsection.

All samples were analysed for Ir and about three dozen other elements by instrumental neutron activation (INAA), with a sensitivity of approximately one part in 10⁹ (1 p.p.b.) for Ir. Radiochemical separations (RNAA) were performed on selected samples to measure Ir in the 10⁻¹² g Ir per g sample range. In none of the 410 samples was Ir detected by INAA (Ir ≤ 1 p.p.b.). The highest values obtained by RNAA were 119 × 10⁻¹² g per g (in a Dunkirk Beach sample) and 112 × 10⁻¹² in a Walnut Creek Gorge sample (Fig. 1). The mean of all Ir values was 50 × 10⁻¹² g per g for the composite New York data set,

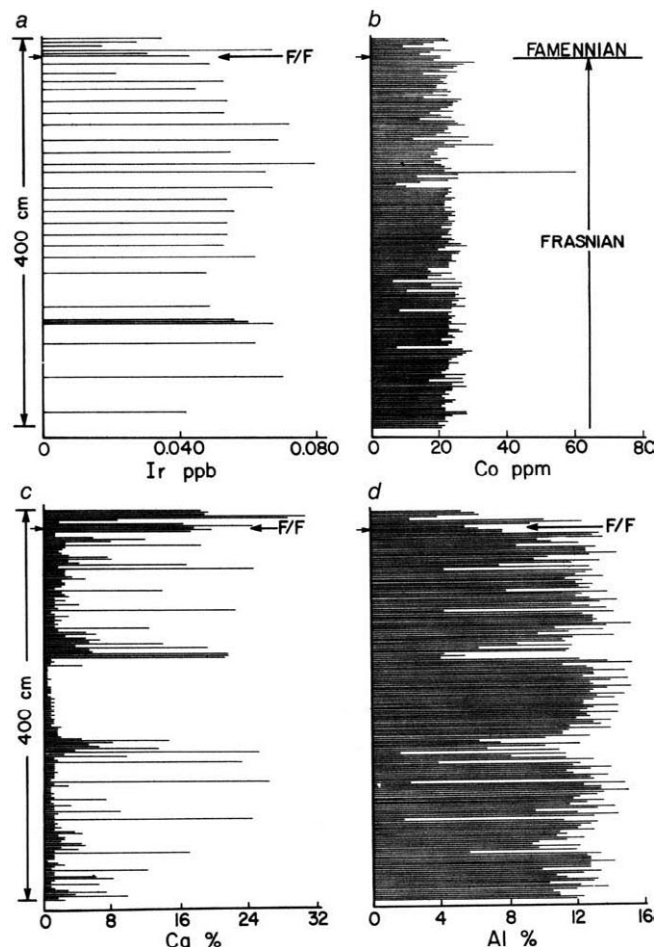


Fig. 2 Variations of Ir (a), Co (b), Ca (c) and Al (d) with depth for the type locality of the Frasnian-Famennian boundary at Sinsin, Belgium. Note that Ca and Al vary sympathetically with carbonate clay proportions.

and the same value for the Belgium section. The only other siderophile elements routinely obtained by INAA were Co and Cr. Plots of these elements against Ir for the combined New York localities and the Belgium locality show only weak correlations. In the Belgium section the highest Ir is 79 × 10⁻¹² g per g. In the Belgian data set Ir shows modest correlations with Al (0.69) and Ca (-0.75). This indicates Ir is associated with higher clay and lower carbonate contents (Fig. 2).

The F-F boundary has been crossed four times in this study without revealing an Ir anomaly. Our sampling interval at Dunkirk Beach, Walnut Creek and Sinsin was on 2-cm centre points, collecting 15 to 20 g of sample. Our INAA method required 5 g samples as opposed to the milligram size quantities used in many INAA laboratories (see for example ref. 21). In order to obtain these large samples it was necessary for us to collect above and below 2-cm centre points such that the actual gap between samples was less than 1 cm. Each sample was homogenized and 5 g irradiated and counted, with a sensitivity of 1 p.p.b. for Ir. For the (higher sedimentation rate) Mills Mills section sampling was on 4-cm centre points also with 15–20 g samples. In this case there was less than 2.5 cm between samples.

Ganapathy²¹ reported an Eocene Ir anomaly in a Caribbean core, and Kyte *et al.*²² reported a Pliocene Ir anomaly in an Antarctic Ocean core. In both cases sedimentation rates (3–10 mm kyr⁻¹ and 3.3 mm kyr⁻¹, respectively) are estimated as the same or slower than for our Walnut Creek and Dunkirk Beach sites. In those cases the Ir anomalies were above our 1 p.p.b. detection limit for 20 and 30 cm of length, respectively. The respective authors speculate that these long lengths of the anomalies may be due to bioturbation (or turbidity currents, or density differences). With similar sedimentation rates in our sites such factors would also operate. In addition, Alvarez *et al.*² illustrate the Ir anomaly for the Italian K-T boundary sections; it is above 1 p.p.b. for 16 cm of length.

Taking all these things into consideration we conclude that our four Devonian sequences, on two continents, had a high probability of locating an Ir anomaly if one were present. We did not require 16, 20, or 30 cm spreads to locate one; 2 to 3 cm would have been sufficient.

Kyte *et al.*²³, Keith²⁴, and Officer and Drake²⁵ have noted the presence of pyrite in the European K-T boundary sections, and the Deep Sea Drilling Project sites. Officer and Drake²⁵ suggest that reducing conditions may play a role in precipitating Ir normally in solution in seawater, creating the observed anomalies. Our New York sections, especially Dunkirk Beach and Walnut Creek Gorge, are pyrite-bearing black shales formed under reducing conditions, yet there is no anomalous Ir observed in them.

The Earth receives periodic impacts from meteorites of the size postulated for the K-T event about every 50 Myr (ref. 26). Smaller objects could produce weaker geochemical effects, as may be the cases with the Eocene and Pliocene Ir anomalies. The problem is the cause-effect relationship with large biotic extinctions. It is our conclusion that the F-F extinction does not appear to have been associated with a large-body impact, unless it were an ice-rich (stone-poor) comet or a highly fractionated (Ir poor) body (eucrite, howardite, angrite, or nakhlite).

The research was supported by NSF grant EAR 81-15518 (G.R.McG.), the US Department of Energy (J.S.G., C.J.O.), and the Chalmers Fund of the Field Museum (E.O.). We thank P. Sartenaer, P. Bultynk (Royal Institute of Science of Belgium), J.D. Knight (Los Alamos National Laboratory) and D. Eatough (Field Museum) for major assistance.

Received 18 October 1983; accepted 12 January 1984.

1. McLaren, D. J. *J. Paleont.* **44**, 801–815 (1970).
2. Alvarez, L. W., *et al.* *Science* **208**, 1095–1108 (1980).
3. Raup, D. M. & Sepkoski, J. J. *Science* **215**, 1501–1503 (1982).
4. McLaren, D. J. *Bull. Geol. Soc. Am.* **94**, 313–324 (1983).
5. McGhee, G. R. *Jr Spec. Pap. Geol. Soc. Am.* **190**, 491–500 (1982).
6. Richard, L. V. *N.Y. State Mus. Sci. Service, Map Chart Ser. No. 24* (1975).
7. McGhee, G. R. *Jr Bull. Geol. Soc. Am.* **88**, 806–808 (1977).
8. Dutro, J. T. *Devonian Biostratigraphy of New York* (eds Oliver, W. N. & Klapper, G.) (International Union of Geol. Sci., Washington, DC, 1981).
9. Chadwick, G. H. *Bull. Geol. Soc. Am.* **46**, 305–342 (1935).
10. Klapper, G. *et al. Mem. Geol. Soc. Am.* **127**, 285–316 (1971).
11. House, M. R. *Acta geol. polon.* **23**, 1–14 (1973).
12. Ziegler, W. *Palaeont. Assoc. Spec. Pap.* **23**, 23–47 (1979).
13. Klapper, G. & Ziegler, W. *Palaeont. Assoc. Spec. Pap.* **23**, 199–224 (1979).
14. House, M. R. *Palaeont. Assoc. Spec. Pap.* **23**, 263–280 (1979).
15. McLaren, D. J. *Spec. Pap. Geol. Soc. Am.* **190**, 477–484 (1982).
16. Pepper, J. F. & deWitt, W. *U.S. Geol. Surv. Oil Gas Invest. Chart No. 37* (1950); *U.S. Geol. Surv. Oil Gas Invest. Map OC-55* (1956).
17. deWitt, W. *Bull. Am. Ass. Petrol. Geol.* **44**, 1933–1939 (1960).

18. Patchen, D. G. & Dugolinsky, B. K. (eds), *Guidebook Middle and Upper Devonian Clastics Central and Western New York State* (US Department of Energy–Eastern Gas Shales Project, Morgantown, West Virginia, 1979).
19. Maynard, J. B. *Am. J. Sci.* **280**, 772–786 (1980).
20. Sartenaer, P. *Bull. Inst. r. Sci. nat. Belg.* **T. 44**, No. 43, 1–36 (1968).
21. Ganapathy, R. *Science* **216**, 885–886 (1982).
22. Kyte, F. T. *et al. Nature* **292**, 417–420 (1981).
23. Kyte, F. T. *et al. Nature* **288**, 651–656 (1980).
24. Keith, M. L. *Geochim. cosmochim. Acta* **46**, 2621–2637 (1982).
25. Officer, C. B. & Drake, C. L. *Science* **219**, 1383–1390 (1983).
26. Wetherill, G. W. & Shoemaker, E. M. *Spec. Pap. Geol. Soc. Am.* **190**, 1–13 (1982).

Triassic sea level change and the Ladinian–Carnian stage boundary

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Time-stratigraphic correlations are difficult in many Middle and Upper Triassic sedimentary sections, either because the sections do not contain diagnostic fossils or because of ambiguities in biostratigraphic resolution. Recently, it has been suggested that some Triassic stage boundaries are associated with eustatic changes in sea level^{1,2}. Here evidence is presented to support that view. Three widely separated examples indicate that the local sea level falls were due to a worldwide change in sea level. At any locality, the response to the global fall in sea level was controlled by the interaction between initial water depth, sedimentation rate and local subsidence rate. In the examples presented here, areas characterized by late Ladinian shallow-water sedimentation show either an erosional unconformity or a seaward shift of facies belts at the Ladinian–Carnian boundary. Late Ladinian slope or basinal settings exhibit shallowing-upward trends, an increase in sedimentation rates, or subtle lithological changes across the boundary. These types of lithostratigraphic relationships can be used to improve correlations in areas where Middle and Upper Triassic sedimentary sections are difficult to date.

The original definitions of the Ladinian and Carnian stages make it difficult to precisely locate the boundary between the two. The Carnian stage was first proposed by von Mojsisovičs³ for a number of rock units in the northern Alps of Austria; no type section was designated. Later, von Bittner⁴ defined the Ladinian stage for Middle Triassic rocks exposed in the Southern Alps of what is now northern Italy—again, no type section was designated. Because of the lack of type sections and unambiguously defined lower limits for each stage, the position of the boundary between the two stages has been open to interpretation and still generates much discussion⁵. Recent work by Krystyn⁶ has refined the definition of the base of the Carnian in the Tethyan area, but extrapolation of this boundary to other areas is still problematic.

In the western Dolomite Alps of northern Italy, near von Bittner's⁴ type area of the Ladinian stage, the Ladinian–Carnian boundary is commonly represented either by an unconformity separating shallow-water carbonates or by a change in sedimentation style in basinal and slope sections^{7–9} (Fig. 1). These relationships suggest exposure and erosion in some platform settings, an increase in sedimentation rates basin-ward of the platform margins, and a shallowing-upward trend in some basinal areas, all indicative of a relative fall in sea level. Similar relationships exist across the Ladinian–Carnian boundary in many areas around the Mediterranean Sea (Fig. 2). The widespread distribution of these changes shows that the postulated drop in sea level affected sedimentation throughout the western Tethyan realm.

Two other widely separated areas, the western United States and locations in the Middle East, also show a relative fall in sea level across the boundary between Middle and Upper Triassic sections. In the western United States a widespread

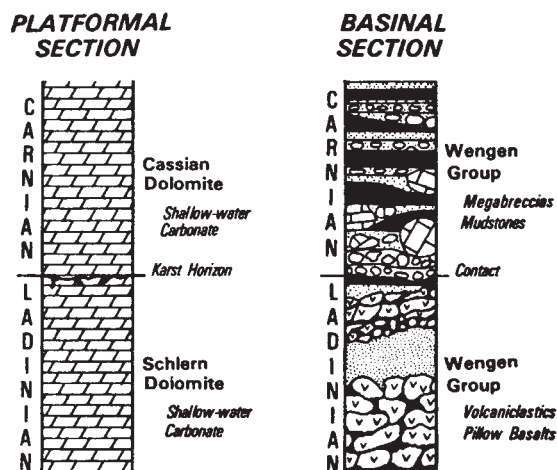


Fig. 1 Stratigraphic sections from platformal and basinal settings in the Alpe di Siusi area of the western Dolomites, northern Italy. In certain platformal areas the Ladinian–Carnian boundary is represented by either an erosion surface or a karst horizon separating shallow-water dolomites. Basinward of some of the platforms, the lower Carnian is represented by megabreccias derived from erosion of the platform margins.

unconformity separates the Upper Triassic non-marine Chinle Formation and its equivalents from underlying Middle and Lower Triassic units. This unconformity is one of the most extensive and easily recognizable unconformities in the western United States¹⁰; it represents a significant erosional relief in many areas, and as much as 58 m of erosional relief has been documented on the unconformity surface in Utah¹¹. The known areal extent of the unconformity is conservatively thousands of square kilometres, and it represents a significant lowering of the erosional base level in the western United States between the middle and late Triassic.

The Middle East provides the third example of a large area where distinct changes caused by a lowering of sea level are observed across the Ladinian–Carnian boundary. Throughout most of this area, the passage from the middle to the late Triassic was characterized either by non-deposition or by a change from shallow-marine deposition to deposition of con-

tinental sediments or carbonate–evaporite sequences¹². In Iraq, for example, the Upper Triassic is believed to rest unconformably on the Middle Triassic everywhere the contact can be observed, although there is no apparent angular unconformity at any locality¹³. In the Atshan No. 1 well in northern Iraq, the Ladinian–Carnian boundary occurs at the contact between the Middle Triassic Geli Khana Formation and the Upper Triassic Kurra Chine Formation. The contact is a haematized and silicified horizon superimposed on leached, vugular dolomite of the Geli Khana Formation¹³. This suggests that the dolomite was subjected to exposure, surface weathering and freshwater dissolution before the deposition of the overlying Kurra Chine Formation.

The local stratigraphic relationships observed across the Ladinian–Carnian boundary have been attributed to tectonic activity in two of the areas used here. Differential uplift and subsidence related to rifting have been cited as explanations of the Middle-to-Upper Triassic stratigraphy of parts of northern Italy and western Austria^{9,14}. The erosional unconformity between the Upper Triassic and underlying rocks in the western United States has been related to broad regional uplift¹⁰. Although there is uncertainty about the amount of time represented by the Ladinian–Carnian boundary in the examples where it is marked by either an erosional unconformity or a hiatus, the available biostratigraphic data indicate that the local sea-level falls were broadly synchronous in all the areas discussed. This, together with the great distances between the areas affected, supports the contention that the local sea-level falls were caused by a eustatic sea-level event.

The magnitude of the eustatic fall is unknown, but because it exposed large areas in several shallow-water settings, the fall must have been in the range of ≥ 10 m instead of just 1 or 2 m. The cause of the eustatic change is also unknown: possible causes include events related to middle and late Triassic rifting in western Europe or as yet unrecognized changes in the Earth's ice budget.

The local responses to the eustatic change depended on several interacting factors, the most important of which were sedimentation rates, subsidence rates and the water depth before the fall. These factors, combined with the eustatic drop in sea level, created predictable lithostratigraphic relationships that can be used to enhance time–stratigraphic correlations in areas where biostratigraphic data are limited or where similar rock types

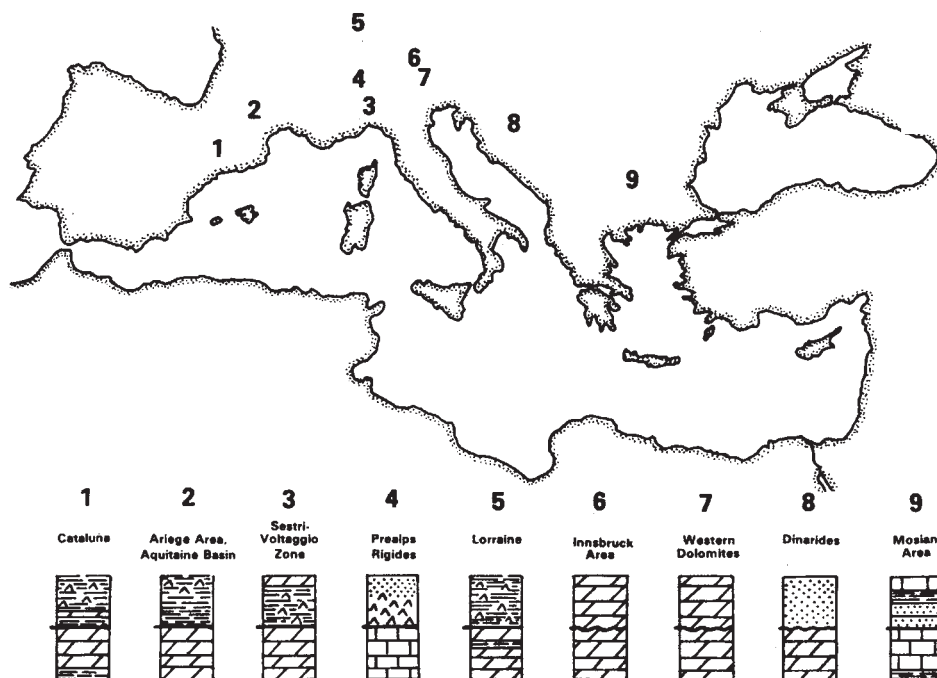


Fig. 2 Selected circum-Mediterranean locations and stratigraphic sections where the Ladinian–Carnian boundary is represented by an erosional unconformity, a hiatus, or an abrupt lithological change. References: Cataluna^{15,16}; Ariège area, Aquitaine Basin¹⁷; Sestri-Voltaggio zone¹⁸; Prealps rigides¹⁸; Lorraine¹⁹; Innsbruck area¹⁴; western Dolomite Alps²⁰; Dinarides²¹; Mosian area²². The stratigraphic columns do not imply thicknesses. Lithological symbols: dashes, shales and mudstones; Δ , evaporites; stippling, sandstones; diagonal slashes, shallow-water dolomites; vertical slashes, shallow-water limestones.

with undiagnostic faunas are superimposed across the Ladinian–Carnian boundary.

I thank Drs P. R. Vail and J. Hardenbol for reading early versions of the manuscript and for discussions. Exxon Production Research Company granted permission to publish this work.

Received 14 November 1983; accepted 13 January 1984.

- Vail, P. R., Mitchum, R. M. Jr & Thompson, S. III. *Mem. Am. Ass. Petrol. Geol.* **26**, 83–97 (1977).
- Vail, P. R. & Todd, R. G. in *Petroleum Geology of the Continental Shelf of North-West Europe* (eds Illing, L. V. & Hobson, G. D.) 216–235 (Institute of Petroleum, London, 1981).
- von Mojsisovič, E. *Geol. Reichsanstalt Jb.* **19**, 91–150 (1869).
- von Bittner, A. *Geol. Reichsanstalt Jb.* **42**, 387–396 (1892).
- Silberling, N. J. & Tozer, E. T. *Am. geol. Soc. Spec. Pap.* **110** (1968).
- Krystyn, L. in *Beiträge zur Biostratigraphie der Tethys-Trias* (ed. Zapfe, H.) 37–75 (Akademie der Wissenschaften, Wien, 1978).
- Bosellini, A. & Rossi, D. in *Reefs in Space and Time* (ed. Laporte, L. F.) 209–233 (Soc. of Econ. Paleontologists and Mineralogists, Tulsa, 1974).
- Assereto, R., Brusca, C., Gaetani, M. & Jadoul, F. *Publ. Ist. Geol. Paleont. Univ. Studi Milano* **231**, 1–34 (1977).
- Bosellini, A., Castellarin, A., Rossi, P. L., Simboli, G. & Sommariva, E. *G. Geol.* **42**, 83–108 (1977).
- Pipiringos, G. N. & O'Sullivan, R. B. *U.S. geol. Surv. Prof. Pap.* 1035–A (1978).
- Davidson, E. S. *U.S. geol. Surv. Bull.* **1229** (1967).
- Sharief, F. A. *J. struct. Geol.* **4**, 299–310 (1982).
- Dunnington, H. V., Wetzel, R. & Morton, D. M. *Lexique Stratigraphique Int. III, Asie*, Fasc. 10a (1959).
- Bradner, R. & Resch, W. in *European Fossil Reef Models* (ed. Toomey, D. F.) 203–231 (Soc. Econ. Paleontologists and Mineralogists, Tulsa, 1981).
- Perez Arlucea, M. & Sopena, A. *Estudios geol.* (in the press).
- Virgili, C. *Boln Inst. geol. min. Esp.* **59**, (1958).
- Burollet, P. *Mem. Bur. Rech. Geol. Min.* **15**, 309–319 (1963).
- Gwinner, M. P. *Geologie der Alpen* (Schweizerbart'sche, Stuttgart, 1978).
- Villemin, J. *Mem. Bur. Rech. Geol. Min.* **15**, 89–122 (1963).
- Bosellini, A. *Abh. geol. Bundesanst., Wien* **34**, 305–323 (1980).
- Herak, M. in *Die Stratigraphie der Alpin-Mediterranen Trias* (ed. Zapfe, H.) 101–106 (Springer, Vienna, 1973).
- Ganev, M. in *Die Stratigraphie der Alpin-Mediterranen Trias* (ed. Zapfe, H.) 93–97 (Springer, Vienna, 1973).

Late Quaternary environments in north-central India

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Microfossil, sediment and oxygen isotope studies of deep-sea cores from the Bay of Bengal and northern Arabian Sea^{1–5} have revealed strong contrasts between high late Pleistocene and low early Holocene salinity values³, indicative of major changes in runoff from the large rivers of southern Asia^{2,3}. Here we present the first radiocarbon-dated continental evidence for late Quaternary changes in river regime and loess accumulation in this region. Our data support earlier palaeoclimatic inferences based solely on oceanic evidence^{2,3}, have important implications for fluvial palaeohydrology⁶, and provide clear environmental constraints against which to test existing regional models of prehistoric land use and early agriculture^{7–9}.

The Son River originates at latitude 23° N in the sandstone uplands of central India and flows eastward along the foot of the Kaimur Hills before it leaves the hills and flows north-eastward to the Ganges (Fig. 1). The Belan is a smaller river with its headwaters in the Kaimur Hills. It flows north and west to the Tons, which joins the Ganges below Allahabad. The Kaimur Hills rise 200–300 m above the Son and Belan valley floors, which are about 150 m above sea level in the surveyed area (Fig. 1). Lower Proterozoic/Vindhyan greywacke sandstones, siltstones, dolomitic limestones and minor igneous and metavolcanic rocks crop out along the Son valley; these are overlain unconformably by the Upper Proterozoic/Vindhyan quartzites, sandstones and shales of the Kaimur escarpment. Tertiary basalts crop out in the Son headwaters, and are the source of the agate, chalcedony and jasper pebbles downstream. Peak flow in both rivers coincides with the summer monsoon. Winter flow is usually low, but a few weeks of rain can make

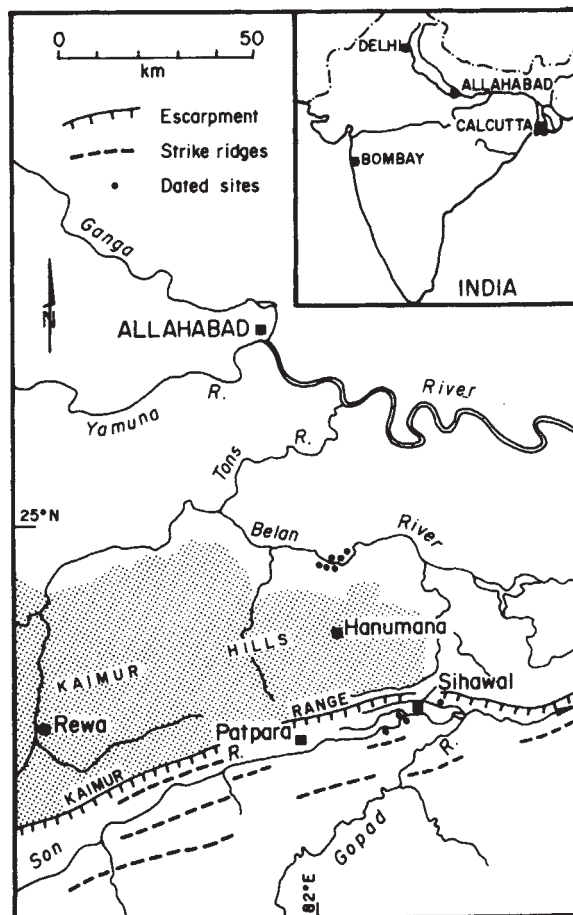


Fig. 1 Location of study area. Black circles denote stratigraphic sections studied in detail.

the Son rise several metres to become a fast-flowing river nearly 1,000 m wide, as in January 1982. The Son is entrenched over 30 m into its late Pleistocene flood plain, the Belan over 20 m. Excellent stratigraphic exposure along river banks and tributary gullies, some of which reach to the foot of the escarpment, allows good three-dimensional reconstruction of late Quaternary alluvial history (Fig. 2).

Time control is based on radiocarbon assays of shell, charcoal and pedogenic carbonate (Table 1). We tested each shell sample

Table 1 Radiocarbon dates for shells, charcoal and carbonate from the Son and Belan valleys, India

	¹⁴ C date (yr BP)	Lab no.	Material
Belan			
A	3,390 ± 100	PRL407*	Charcoal
B	10,030 ± 115	SUA1421	Shell
C	18,055 ± 150	Beta 4789	Shell
	19,715 ± 340	TF 1245*	Shell
	25,430 ± 350	Beta 4877	Shell
D	25,790 ⁺⁸³⁰ ₋₇₆₀	PRL 86*	Shell
	26,270 ± 660	Beta 4790	Shell
Son			
E	3,215 ± 70	Beta 4879	Shell
	4,130 ± 110	Beta 6414	Charcoal
	4,740 ± 80	Beta 6415	Shell
F	5,305 ± 90	SUA 1422	Carbonate†
G	11,870 ± 120	Beta 4792	Shell
H	13,145 ± 140	SUA 1420	Carbonate†
I	20,135 ± 220	Beta 4791	Shell
J	26,250 ± 420	Beta 4793	Shell

* Ref. 7.

† Post-depositional, and so a minimum age only.

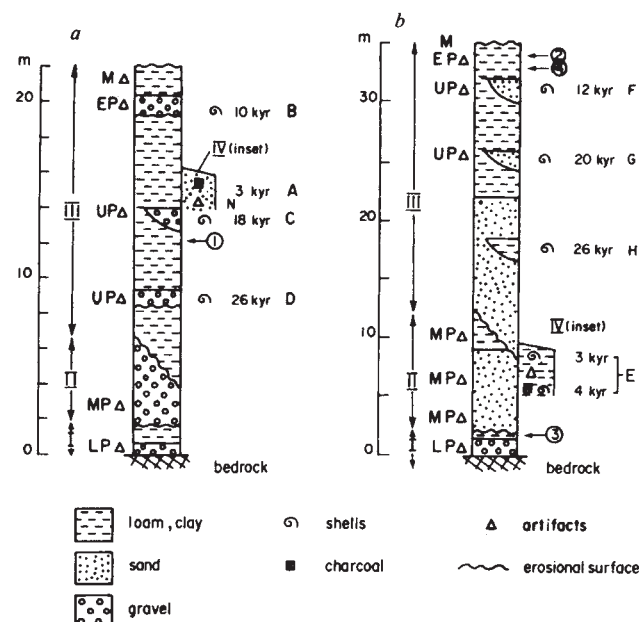


Fig. 2 Composite Quaternary geological sections for the Belan (a) and middle Son (b) valleys, where alluvial formations III and IV are correlated by radiocarbon ages and I and II are provisionally correlated by lithofacies and prehistoric artefact assemblages. Ages are given in thousands of years; A–J refer to Table 1. LP, MP, UP and EP denote Lower, Middle, Upper and Epi-Palaeolithic stone tool assemblages found in primary context. M, Mesolithic; N, Neolithic. Arrowed numerals 1–4 indicate the stratigraphic level of loess samples shown in Fig. 3.

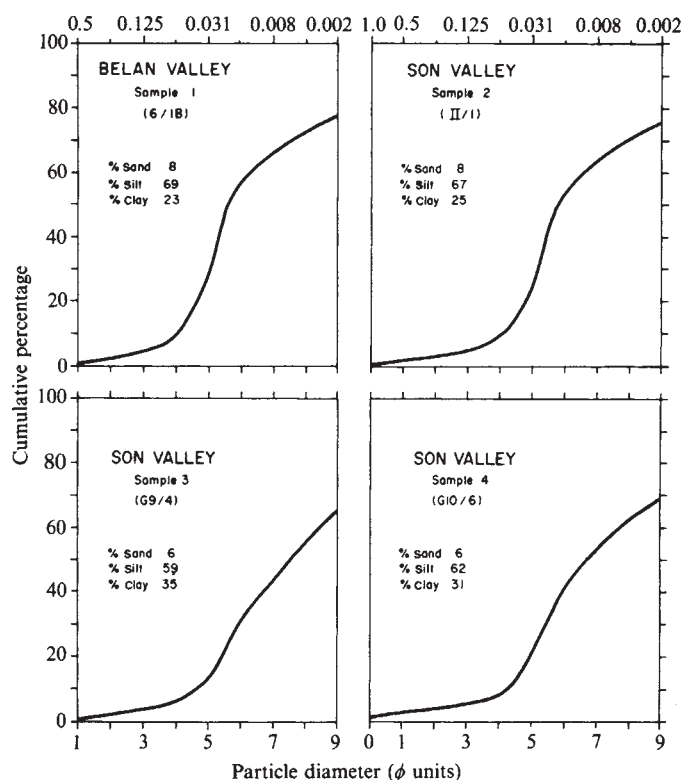


Fig. 3 Particle size distribution of four loess samples from the Belan and Son valley study areas. Stratigraphic levels shown in Fig. 2. The phi unit scale is based on $\phi = -\log_2 \text{mm}$. Analyses taken down to $\phi = 9$.

for recrystallization by using X-ray diffraction calibrated against known quantities of calcite and aragonite. No sample showed any sign of recrystallization from aragonite to calcite. As a further test we dated molluscs from the present Belan River.

The two oldest alluvial formations (Fig. 2, I and II) seen exposed in the Son and Belan valleys contain Acheulian and Middle Palaeolithic artefact assemblages, and are beyond the present range of ^{14}C -dating. The two youngest formations (Fig. 2, III and IV) are now securely dated in both valleys, one to late Pleistocene, including the last glacial maximum (~25 kyr to 10 kyr), the other to late Holocene (~5 kyr to 3 kyr).

In the middle Son valley the 25–10-kyr alluvium mantles the older formations and forms a plain 5–10 km wide and over 100 km long on either side of the river. The plain is 30–35 m above the low-water bed of the Son, which includes bedrock, sand and gravel reaches. Near the river two alluvial members are evident in formation III: a lower sandy member 10–15 m thick, with carbonate-cemented gravel lenses sometimes rich in mammal fossils, and an upper horizontally-bedded clay member also 10–15 m thick, with thin sandy interbeds⁶. The lower sandy member lenses out 1–2 km away from the river. Towards the hills, formation III grades into a massively-bedded fine sandy clay which we interpret as loess. For up to 10 m in vertical section the clay is of uniform colour, mineralogy and grain size (Fig. 3). The fine sand fraction consists mainly of sub-rounded to sub-angular quartz; the silt fraction, also mainly quartz, is angular and resembles silt grains produced in recent wind abrasion experiments¹⁰. Away from the piedmont the loess was subjected to erosion and redeposition by the river and its major tributaries.

The middle reaches of the Belan are entrenched into late Pleistocene loess with interbeds of shell-bearing carbonate and ironstone gravels up to 1 m thick. Unbroken freshwater mollusc shells from these gravels range from a basal age of 26 kyr through 18 kyr to 10 kyr (Fig. 2). Aeolian deposition of the loess was thus interrupted by minor phases of erosion and, in places, by extensive fluvial reworking¹¹.

Sporadic deposition of wind-blown dust continued during the early Holocene Mesolithic occupation^{7,8} of both valleys, but with a colour change locally from yellow-brown to black, possibly indicative of organic staining of dust trapped in seasonal or perennial swamps. From about 10–8 kyr until about 5–4.5 kyr, the two rivers incised 20–30 m into their former flood plains, exposing older alluvial formations. Towards 4.5 kyr until roughly 3 kyr, the Son and Belan aggraded once more, but only to about +12 m, and not +30 m, as in the terminal Pleistocene. Neolithic cultural remains^{7,9} locally abound within the sands and clays of this late Holocene alluvium, indicating that the Son and Belan valleys have been farmed for at least 4,000 yr.

The closest probable source area for the late Pleistocene loess is the alluvial plain of the Ganges which extends from the Himalayan foothills to within 50 km of the Belan and 100 km of the Son (Fig. 1). Himalayan pine pollen within the loess also points to a northern provenance (Dr Vishnu Mittre, personal communication). The Thar Desert 900 km to the WNW may also have furnished some dust. Accumulation of late Pleistocene loess within sheltered valleys like the Son and Belan accords with Duplessy's inference from salinity patterns in the Bay of Bengal that at the last glacial maximum, strong dry winds blew from the north-east during winter³.

A change from sparse vegetation and high sediment yields in the Son headwaters at glacial maximum (25 to ~17 kyr), to dense vegetation and low sediment yields during the warm, wet postglacial is consistent with the observed change in alluvial sedimentation⁶. The cross-bedded sands of Son formation III are capped by overbank clays, indicating a change from bedload to suspension load. Early Holocene incision into the Pleistocene flood plain implies excess potential energy, a low load/discharge ratio, and an efficient channel cross-section of low width/depth ratio.

Here, as elsewhere¹², a well drained flood plain and reliable rains offered Neolithic farmers a chance of success. Farming might have been impossible during the last glacial maximum,

when summer and winter monsoonal rains were infrequent and unreliable.

We thank Professors J. D. Clark and G. R. Sharma for inviting us to work with them; V. D. Misra, D. Mandal, B. B. Misra, J. N. Pal and Umesh Chattopadhyaya for field guidance; the Smithsonian Institution and Macquarie University for funds; and K. Royce for help in 1980.

Received 25 October; accepted 18 January 1984.

1. Prell, W. L. *et al.* *Quat. Res.* **14**, 309–336 (1980).
2. Cullen, J. L. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **35**, 315–356 (1981).
3. Duplessy, J. C. *Nature* **295**, 494–498 (1982).
4. Van Campo, E., Duplessy, J. C. & Rossignol-Strick, M. *Nature* **296**, 56–59 (1982).
5. Kolla, V. & Biscaye, P. E. *J. sedim. Petrol.* **47**, 642–649 (1977).
6. Williams, M. A. J. & Royce, K. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **38**, 139–162 (1982).
7. Sharma, G. R., Misra, V. D., Mandal, D., Misra, B. B. & Pal, J. N. *Beginnings of Agriculture*, 1–200 (Abinash Prakashan, Allahabad, 1980).
8. Sharma, G. R. & Clark, J. D. (eds) *Palaeo-environments and Prehistory in the Middle Son Valley, Madhya Pradesh, North-Central India* 1–320 (Abinash Prakashan, Allahabad, 1983).
9. Clark, J. D. & Williams, M. A. J. in *The Prehistory of India* (ed. Jacobson, J.) (American Institute for Indian Studies, Washington DC, in the press).
10. Whalley, W. B., Marshall, J. R. & Smith, B. J. *Nature* **300**, 433–435 (1982).
11. Mujumdar, G. G. & Rajaguru, S. N. in *Studies in Indian Archaeology* (eds Deo, S. B. & Dhavalikar, M. K.) 96–105 (Indian Antiquary, Bombay, 1970).
12. Vita-Finzi, C. in *The Domestication and Exploitation of Plants and Animals* (eds Ucko, P. J. & Dimbleby, G. W.) 31–34 (Duckworth, London, 1969).

Active intra-basinal highs and palaeodrainage reversals in the late orogenic hominoid-bearing Siwalik basin

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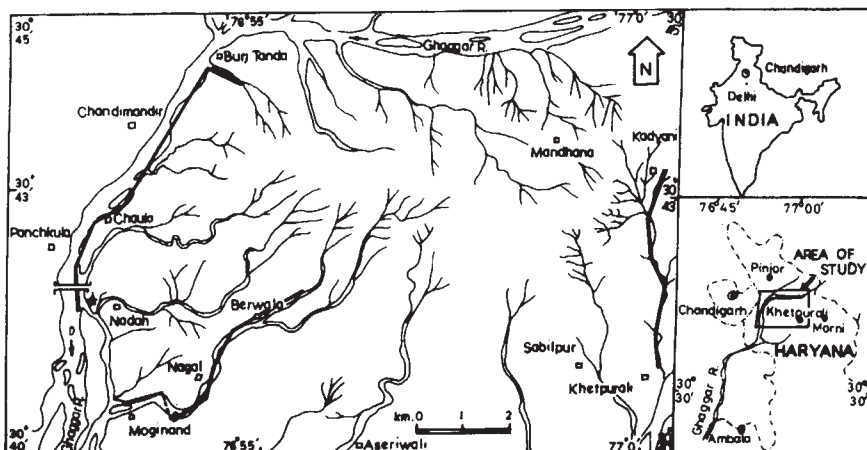
The Himalayan mountain chain is a product of the continent-to-continent collision of India with Asia. Major mountain building activity in the Himalaya was followed by the shedding of sediments which were deposited in extensive non-marine basins on the Indian craton. The late orogenic hominoid-bearing Siwalik basin, comprising alluvial deposits, occurs along the northern margin of the Indian plate and has itself been uplifted. Major Siwalik palaeodrainages are believed to have originated in the Himalaya. We now report bed by bed measurement of palaeocurrent data of the Upper Siwalik rocks from the outermost Foothills Belt which suggest that active intra-basinal highs have locally caused palaeodrainage reversals. Discontinuity in apparently continuous stratigraphical sequences is recognized as the composite aggradation of alternating depositional events related to drainages controlled by basin margin and intra-basinal structural highs. The period of repeated uplift of these structures is estimated to be 1 to 5×10^5 yr.

Basinal evolution and the nature of the lithic fill in late orogenic basins are determined largely by contemporaneous tectonic activity^{1–3}. It is therefore reasonable to hypothesize that palaeoflow variability recorded in the deposits of late orogenic basins may be a manifestation of various episodes of tectonic activity. It has been shown that, taken as a time sequence, the vectorial properties of sedimentary rocks give useful information about regional tectonic events⁴. In contrast, within a single bed the vectors provide palaeogeographic information. The main conclusion drawn from the meagre data available^{5–9} on the palaeocurrents and dispersal patterns of the Siwalik late orogenic deposits is that the palaeodrainage was from the orogenic highlands in the north.

In the Foothills Belt near Chandigarh (Fig. 1), the Upper Siwalik Subgroup, containing rich vertebrate mammalian fauna^{10–13} and hominoid remains^{14,15}, are being intensively investigated. The temporal variability in bed by bed directional data of the Upper Siwalik Subgroup indicates the presence of four sediment-vector sequences in the Khetpurali section (K_a , K_b , K_c and K_d) and three sediment-vector sequences in the Ghaggar section (G_a , G_b and G_c) (Figs 1, 2). The palaeodrainage pattern in the Khetpurali section reveals that north-flowing drainage had a major influence twice in the stratigraphical column within a 1–3 Myr interval. By inference, the sediment-vector sequences K_a and K_c (Fig. 2) indicate the periodic predominance in tectonic activity of intrabasinal growth structures (folds) recognized independently through geophysical (seismic and gravity) data^{16,17}. The subsurface synclinal structure near Ambala consists of older Tertiary rocks of the molassic phase, with a sediment thickness of ~3,000 m (ref. 16).

There is also a mutually antipathetic temporal relationship between polycrystalline and monocrystalline quartz at the level corresponding to the drainage reversal from sediment-vector sequence K_a to K_b (Fig. 2). In contrast to this, in the Ghaggar section 13 km away, the drainage originating from the intrabasinal highs seems to have had no effect. The sediment-vector sequences in the Ghaggar section can be related to the periodic activities of adjacent basin-margin structural highs (Fig. 3) in the north. Geological data reveal that in both the north-east and north-west, topographic 'highs' exist to the north of the Main Boundary Fault and form part of the set of imbricate thrust structures in the outer part of the Lesser Himalaya bordering the Siwalik basin (Fig. 3). The differences in the sediment dispersal patterns of the two neighbouring sections indicate that the influence of the intrabasinal high may be of local importance. This, however, does not exclude the possibility of the input of fine sediments from the south in the Ghaggar section. The concept of 'competitive drainage' at the depositional sites as related to the episodicity of basin marginal and intrabasinal structure has emerged. In the Khetpurali section, the competition is between drainage emanating from a tectonic high in the north and from an intra-basinal tectonic high located in the opposite direction in the south. The competition in the

Fig. 1 Map of the area investigated. Measured sections are marked by thick lines.



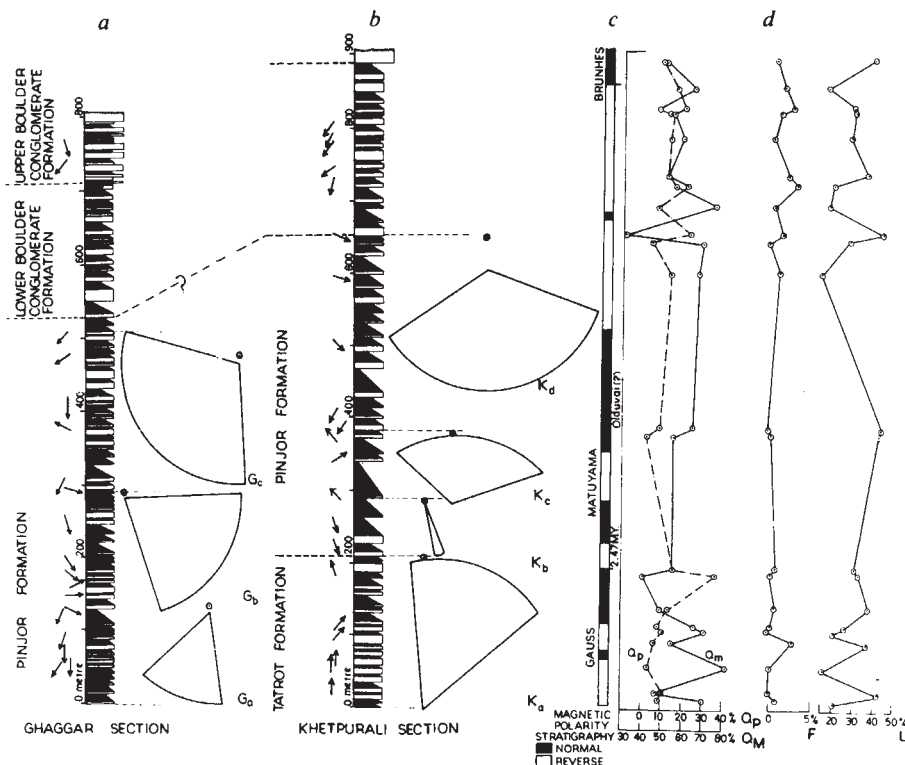
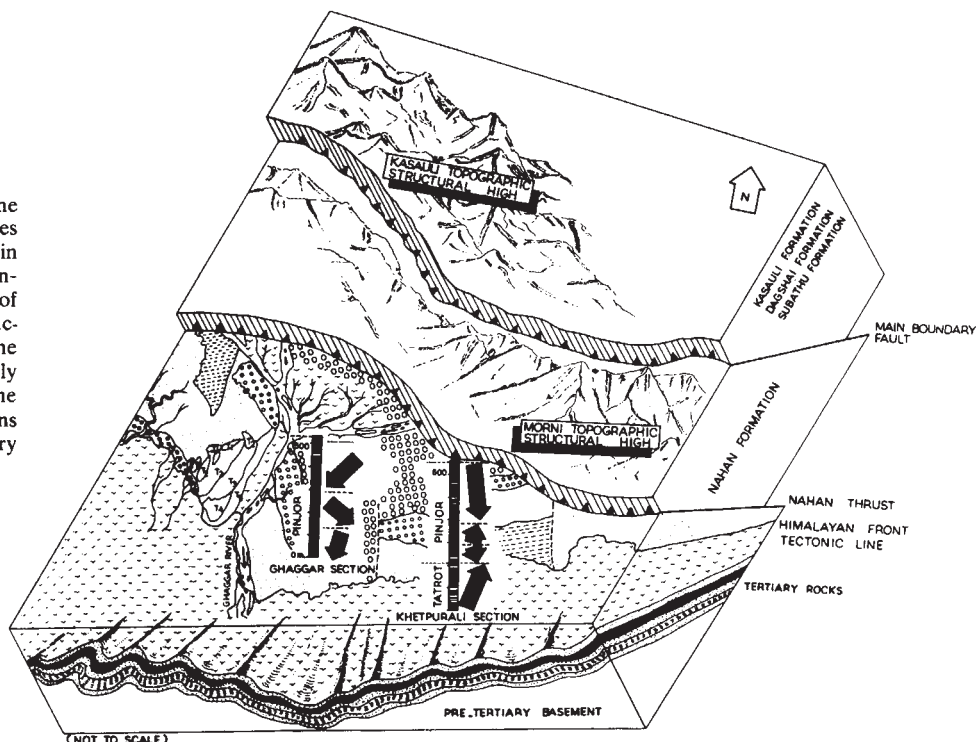


Fig. 2 *a, b*, Stratigraphical logs of the Ghaggar and Khetpurali sections showing the positions of the sediment-vector sequences (G_a , G_b , G_c and K_a , K_b , K_c , K_d). Arrows to the left of the logs indicate palaeoflow vectors for individual sandstone and conglomerate bodies. *c*, Magnetic polarity zonation of the Khetpurali section. *d*, Relative percentages of polycrystalline quartz (Q_p), monocrystalline quartz (Q_m), feldspar (F) and lithic fragments (L) in the Khetpurali stratigraphical column.

Fig. 3 Perspective diagram showing the subsurface basement synclinal structures in the south and topographical highs in the north which control the palaeodrainages of the sediment-vector sequences of the Upper Siwalik Subgroup. Major structural planes are shown in the diagram. The Nahani Formation represents the early phase of molassic sedimentation. The Kasauli, Dagshai and Subathu formations represent the pre-molassic early Tertiary sedimentary sequence.



Ghaggar section is, however, between two adjacent basin-marginal structures (Fig. 3).

Using the magnetic polarity stratigraphical data of the Khetpurali section¹⁸, we estimate that the sediment-vector sequences K_a , K_b and K_c span time intervals of 4.4×10^5 , 1.4×10^5 and 1.8×10^5 yr, respectively. The average estimate of 50 cm per 1,000 yr for the rate of sedimentation¹⁹ of the Upper Siwalik Subgroup suggests time intervals of 2.7×10^5 , 3.3×10^5 and 4.3×10^5 yr for the sediment-vector sequences G_a , G_b and G_c of the Ghaggar section. It therefore seems reasonable to conclude that the period of activity of basin-marginal and intra-basinal structural highs is of the order of 1 to 5×10^5 yr.

Thus, active secondary sources related to intra-basinal structural highs may be locally important and result in discontinuities

in apparently continuous late orogenic stratigraphical sequences. This is of paramount importance in palaeoecological reconstructions of the hominid/hominoid sites of the Siwalik basin.

We thank Paramjit Singh for help with the diagrams. Professor I. P. Martini offered critical comments on an earlier version of the manuscript. R. K. was supported by a UGC grant.

Received 13 December 1983; accepted 21 February 1984.

1. Van Houten, F. B. *Science* **166**, 1506–1508 (1969).
2. Van Houten, F. B. in *Modern and Ancient Geosynclinal Sedimentation* Vol. 19 (eds Dott, R. H. Jr & Shaver, H.) 260–273 (Society Ecology, Paleontology and Mineralogy Spec. Publ. Oklahoma, 1974).
3. Miall, A. D. *Can. J. Earth Sci.* **15**, 1613–1632 (1978).
4. Tanner, W. F. *The Mountain Geologist* **8**, 151–153 (1971).
5. Tandon, S. K. *Himalayan Geol.* **1**, 59–74 (1971).
6. Parkash, B., Bajpai, I. P. & Saxena, H. P. *Geol. Mag.* **111**, 1–14 (1974).

7. Parkash, B., Goel, R. K. & Sinha, P. *J. geol. Soc. Ind.* **16**, 337-348 (1975).
8. Parkash, B., Sharma, R. P. & Roy, A. K. *Sedim. Geol.* **25**, 127-159 (1980).
9. Qidwai, H. A. & Swarnkar, B. M. *J. Ind. Ass. Sedimentologists* **2**, 33-40 (1980).
10. Sahni, M. R. & Khan, E. *Res. Bull. Panjab Univ.* **12**, 263-264 (1961).
11. Sahni, M. R. & Khan, E. *J. palaeont. Soc. India* **4**, 61-74 (1964).
12. Nanda, A. C. *Curr. Sci.* **42**, 319 (1973).
13. Azzaroli, A. & Napoleone, G. *Riv. ital. Paleont. Stratigr.* **87**(4), 739-762 (1982).
14. Sharma, J. C. *Curr. Anthropol.* **18**, 94 (1977).
15. Kennedy, K. A. R. *Proc. int. Workshop—Late Cenozoic Paleoclimatic Changes in Kashmir and Central Asia* (Physical Research Laboratory, Ahmedabad, 1982).
16. Balakrishna, T. S. & Choudhry, S. K. *Geol. Surv. Ind. Misc. Publs* **41**, 175-182 (1979).
17. Aditya, S., Raju, A. T. R. & Shukla, S. N. *Geol. Surv. Ind. Misc. Publs* **41**, 127-140 (1979).
18. Tandon, S. K., Kumar, R., Koyama, M. & Niitsuma, N. *J. geol. Soc. Ind.* **25**, 45-55 (1984).
19. Opdyke, N. D. *et al. Paleogeogr., Paleoclimatol., Palaeoecol.* **27**, 1-34 (1979).

Denervation releases a neuronal survival factor in adult rat hippocampus

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Target-derived macromolecules in the peripheral nervous system apparently play an important part in the regulation of neuronal survival and maturation during critical stages of development¹⁻⁷. Similar trophic factors could also be required in the central nervous system (CNS) for the maintenance of intracerebral connections, and neuronal cell death may result from a decrease or failure of a target-derived trophic support^{8,9}. Recent experiments have demonstrated the presence of factors in brain extracts or astrocytic glial cells which can support the survival of embryonic sympathetic, parasympathetic or sensory ganglionic neurones *in vitro*¹⁰⁻¹⁴. The importance of these putative factors for neuronal survival *in vivo* has, however, so far not been demonstrated. In the study reported here we have used the intracerebral grafting technique to monitor the availability of survival factors in an adult CNS target area and show here that neonatal rat superior cervical cells, which have an absolute requirement for such factors for their survival^{1,2,3,15}, will survive in the hippocampal formation of adult rats only in the presence of a denervating lesion.

In the first experiment, an aspirative lesion was made unilaterally in the retrosplenial cortex in 19 adult female Sprague-Dawley rats, as described previously¹⁶ (Fig. 1). This lesion exposes the hippocampus posterodorsally, and transects at the same time the dorsal subiculum and the perforant path axons innervating the dorsal hippocampus and dentate gyrus. In the same operation the superior cervical ganglia (SCG) were excised from 1-day-old rat pups and implanted (two in each cavity) in direct contact with the exposed hippocampal surface. The cavity was filled with gel-foam (Spongostan; Ferrosan, Denmark) and the wound was closed. In 10 of the 19 implanted animals a second aspirative lesion was made, in the same surgical session, through the ipsilateral fimbria-fornix bundle (Fig. 1). This lesion transects major afferent and efferent projections of the hippocampal formation, including the cholinergic septal afferents¹⁷. Seven weeks after surgery the rats were perfused and processed for catecholamine fluorescence histochemistry, according to the ALFA method for paraffin-embedded specimens¹⁸. Serial 15- μ m sections were cut coronally through the implant and the adjacent hippocampal formation, and surviving ganglionic cell bodies were counted in every third section in the fluorescence microscope according to Abercrombie¹⁹.

The survival of the implanted SCG neurones is summarized in Table 1. The implanted neurones survived very poorly in the rats with intact fimbria-fornix. In fact, not more than one fluorescent SCG neurone survived in any of the rats in this group (Fig. 2a). By contrast, all implants in the fimbria-fornix lesion group possessed large numbers of surviving SCG neurones. These neurones occurred singly or in clusters on the surface of the host hippocampus or dentate gyrus. They were

seen to extend processes into the host hippocampal formation where they ramified extensively, above all in the hilar zone of the dentate gyrus (Fig. 2b-d). The number of surviving neurones (average of 90 neurones per two implanted ganglia) was about one-sixth of that previously recorded for implants of adult SCG (average of 145 per half ganglion)¹⁷.

In addition to the ganglionic neurones, clusters of the chromaffin-like, so-called SIF cells were present in the implants. These SIF clusters occurred in both groups of animals and their survival seemed to be independent of the fimbria-fornix lesion.

In a second experiment we sought to quantify biochemically the total noradrenaline (NA) content in the transplant including its axonal processes in the target tissue. Twelve animals were initially treated with 6-hydroxydopamine (6-OHDA) and bilateral sympathectomy to eliminate endogenous noradrenaline, and subsequently received neonatal ganglia transplants to the retrosplenial cavity with or without simultaneous fimbria-fornix lesion as above. The results are presented in Table 2.

In the graft plus hippocampus target combined, no transplant-derived noradrenaline was detected in rats without fimbria-fornix lesions (group 3 in Table 2). By contrast, transplant-derived noradrenaline was about 30 times greater when the neonatal SCG transplants were grafted in the presence of the fimbria-fornix lesion (group 4). In fact, the content of transplant-derived noradrenaline in the transplant and target tissue in the presence of target denervation was equal to the normal endogenous noradrenaline content of the target tissue (group 1), while in the absence of target denervation there appeared to be no significant transplant-derived noradrenaline in the transplant or the target tissue. The average total noradrenaline content measured in the presence of the denervating lesion can be estimated to correspond to the content of approximately 50 adult rat SCG neurones. This value was calculated from the data of Dahlström²⁰, who estimated the average total noradrenaline content in rat SCG neurones (cell body plus total axonal network) at approximately 250 pg per neurone. The average total noradrenaline content in the SCG graft and hippocampal target combined was about 13 ng in the denervated animals.

Fimbria-fornix transection, or lesion of the septal-diagonal band area, has previously been shown to stimulate the growth of adrenergic ganglionic fibres into the denervated hippocampus^{17,21-23} as well as the regeneration of the intrinsic adrenergic locus coeruleus axons after chemically induced axotomy¹⁷. Both these effects have been proposed to be due to the release of a neurotrophic factor (or factors) whose production normally is under the control of the cholinergic septal afferents^{17,21,22}. In the present study the survival of the implanted neonatal SCG neurones depended on the integrity of the fimbria-fornix bundle,

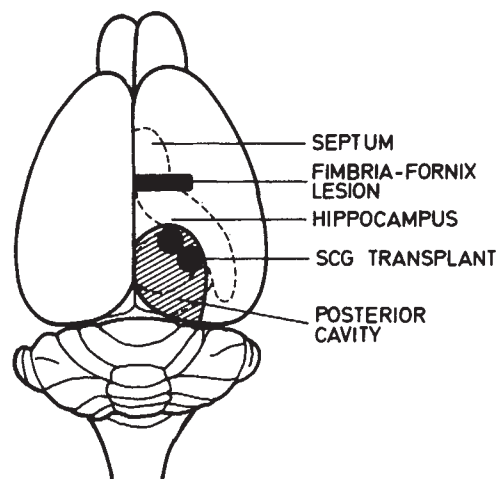


Fig. 1 Schematic representation of retrosplenial cortex cavity, neonatal superior cervical ganglia placement in the cavity, and the location of the aspirative lesion through the ipsilateral fimbria-fornix.

Table 1 Neonatal superior cervical ganglion cells surviving in the brain following transplantation

Group I: Transplant with fimbria-fornix transection		Group II: Transplant without fimbria-fornix transection	
Rat no.	Fluorescent SCG cells*	Rat no.	Fluorescent SCG cells*
2	54	1	0
3	95	4	0
5	84	6	0
8	72	7	0
10	68	9	1
12	58	11	0
15	50	13	1
16	171	14	1
18	113	17	0
19	137		
<i>n</i> = 10	$\bar{x}$ = 90.2	<i>n</i> = 9	$\bar{x}$ = 0.3

* Calculated according to the method of Abercrombie¹⁹.

which suggests that the hippocampal target factor (or factors) released by deafferentation can provide a nerve growth factor (NGF)-like support for ganglionic neuronal survival. This effect seems to be specific for afferents running in the fimbria-fornix (presumably the septal cholinergic projection) since the aspirative lesion of the retrosplenial cortex, which denervates the dorsal hippocampal formation of its massive perforant path input, did not have the same effect. Moreover, since the SCG implants in the present study were placed in the cavity outside the host hippocampus, we propose that the trophic support of the implanted neurones is due to the release of diffusible survival factor(s). Nieto-Sampedro *et al.*¹⁴ have previously shown that factors with such properties indeed accumulate in a cortical wound of the type used in the present study.

In the peripheral nervous system there is good evidence that target-derived neuronotrophic factors have an important role in the regulation of survival, development and maintenance of

Table 2 Transplant-derived NA content in animals with and without fimbria-fornix transection

	NA content (ng per 100 µg protein) (transplant + hippocampus combined)
(1) Normal hippocampus	0.231 ± 0.004
(2) 6-OHDA + bilateral sympathectomy	0.017 ± 0.005
(3) 6-OHDA + bilateral sympathectomy with SCG transplant	0.009 ± 0.001
(4) 6-OHDA + bilateral sympathectomy with SCG transplant and fimbria-fornix lesion	0.287 ± 0.003

Differences: (4) versus (3) = $P < 0.001$; (4) versus (1) = $P < 0.025$; (3) versus (2) = n.s. (one-way analysis of variance with *post hoc* Newman-Keuls test).

For this experiment 12 rats were depleted of all CNS noradrenaline (NA) by 6-OHDA (250 µg in 20 µl) injected into the right lateral ventricle 1 month before transplantation, and bilateral sympathectomy 5 weeks before transplantation. All 12 rats received implants of two neonatal ganglia in the retrosplenial cavity as in the previous experiment. Six of these rats received simultaneous unilateral fimbria-fornix lesions ipsilateral to the transplant. An additional group of normal unoperated rats was taken for controls. Noradrenaline in the transplant and hippocampal formation combined was assayed radioenzymatically by a modification³¹ of the method of Da Prada and Zurcher³².

sympathetic adrenergic neurones¹⁻³, parasympathetic cholinergic neurones³⁻⁵ and spinal mononeurones^{6,7}. NGF appears to have this role for adrenergic ganglionic neurones, whereas non-NGF-like factors are likely to act on the cholinergic autonomic or spinal neurones. Naturally occurring cell death has been suggested to be due to a competition within the innervating neuronal population for trophic support from the target^{1-5,24,25},

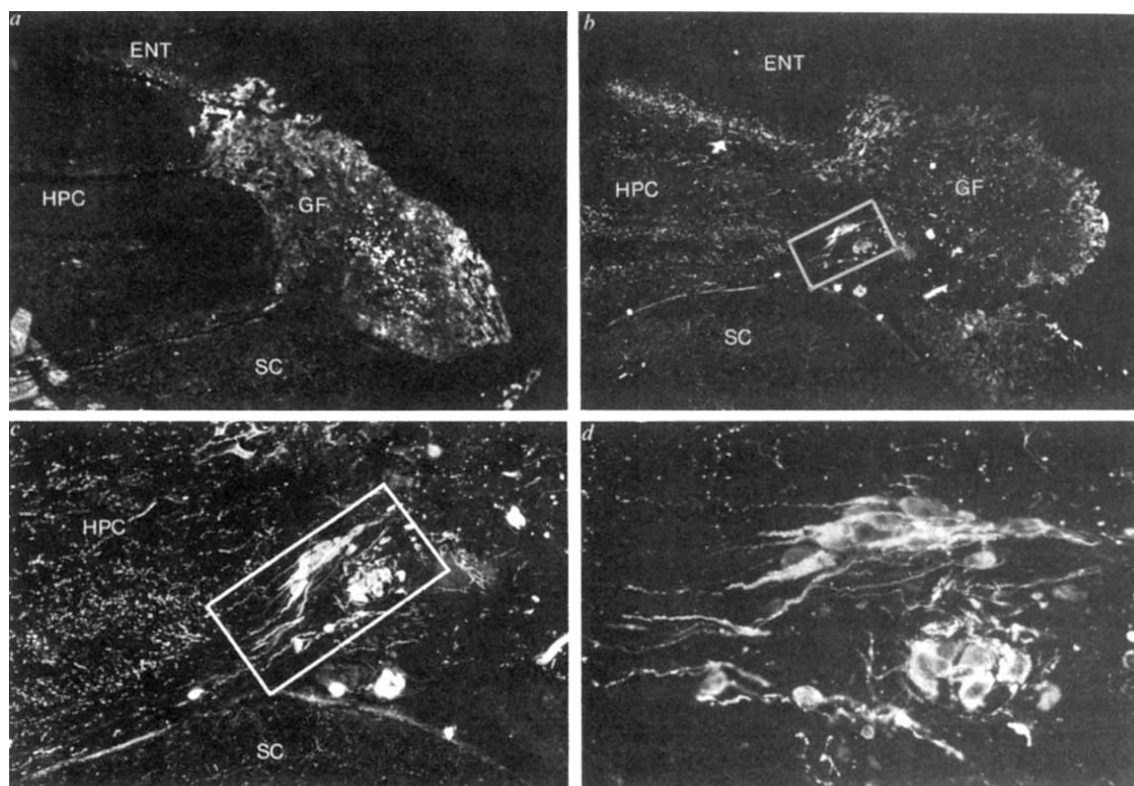


Fig. 2 Fluorescence photomicrographs of retrosplenial cortex cavity 7 weeks after neonatal SCG transplant. *a*, In the absence of ipsilateral fimbria-fornix lesion; *b*, in the presence of ipsilateral fimbria-fornix lesion; *c*, higher magnification of *b*; *d*, higher magnification of inset in *b*. HPC, hippocampal formation; GF, gel-foam; ENT, entorhinal cortex; SC, superior colliculus. *a* and *b*, $\times 20$; *c*, $\times 50$; *d*, $\times 100$.

and, consequently, maintenance of the innervating neurones throughout the lifespan of the animal may depend on the same mechanism. The present observations provide evidence for the *in vivo* elaboration of neuronal survival factors from target areas within the CNS and, as has been reported in peripheral targets^{6,7,23,26}, their production can be greatly increased after partial denervation of the target. This is consistent with the idea that target-derived trophic factors are important also for the maintenance of intracerebral connections.

The survival factor or factors monitored in the present experiments may be particularly relevant with respect to the maintenance of the septal and basal forebrain cholinergic projection system and the locus coeruleus adrenergic projection system, both of which have been shown to be involved in the neurodegenerative processes associated with normal or pathological aging²⁷⁻²⁹. Interestingly, Pearson *et al.*³⁰ have recently

pointed out that extensive retrograde neuronal shrinkage and degeneration, similar to that seen in patients with Alzheimer's disease, also occur in the nucleus basalis and locus coeruleus after extensive cortical damage, that is, after lesions that would deprive the cholinergic and noradrenergic neurones of a potential source of target-derived trophic support. The intracerebral neural implantation technique should provide a valuable tool as an *in vivo* assay system for the further exploration of cerebral neurotrophic mechanisms and their changes after damage and with age²⁷⁻²⁹.

We thank Ragnar Mårtensson for assistance with the photography, and Jan Berglund, Kerstin Fogelström, Gertrude Stridsberg and Ulla Jarl and Jan Berglund for technical assistance. This work was supported by grants from the Swedish MRC and from the Kock and Syskonen Svensson Foundation, and NIA grant AG03766.

Received 5 August 1983; accepted 8 February 1984.

- Hendry, I. A. *Rev. Neurosci.* **2**, 149-194 (1976).
- Black, I. B. A. *Rev. Neurosci.* **1**, 183-214 (1978).
- Varon, S. & Bunge, R. P. A. *Rev. Neurosci.* **1**, 327-361 (1978).
- Landmesser, L. & Pilar, G. *Fedn. Proc.* **37**, 2016-2022 (1978).
- Nishi, R. & Berg, D. K. *Nature* **277**, 232-234 (1979).
- Henderson, C. E., Huchet, M. & Changeux, J. P. *Nature* **302**, 609-611 (1983).
- Hill, M. A. & Bennett, M. R. *Neurosci. Lett.* **35**, 31-35 (1983).
- Appel, S. H. *Ann. Neurol.* **10**, 499-505 (1981).
- Hefli, F. *Ann. Neurol.* **13**, 109 (1983).
- Lindsay, R. M. *Nature* **282**, 80-82 (1979).
- Norrgren, G., Ebendal, T., Bewley, M., Jacobson, C.-O. & Porath, J. *Expl. Cell Res.* **130**, 31-38 (1980).
- Crutcher, K. A. & Collins, F. *Science* **217**, 67-68 (1982).
- Barde, Y. A., Edgar, D. & Thoenen, H. *EMBO J.* **1**, 549-553 (1982).
- Nieto-Sampedro, M. *et al. Science* **217**, 860-861 (1982).
- Levi-Montalcini, R. & Angeletti, P. V. *Physiol. Rev.* **48**, 534-568 (1968).

- Gage, F. H., Björklund, A. & Stenevi, U. *Nature* **303**, 819-821 (1983).
- Björklund, A. & Stenevi, U. *Brain Res.* **229**, 403-428 (1981).
- Loren, J., Björklund, A., Falck, B. & Lindvall, O. *J. Neurosci. Meth.* **2**, 277-300 (1980).
- Abercrombie, M. *Anat. Rec.* **94**, 239-247 (1946).
- Dahlström, A., thesis, Karolinska Institutet, Stockholm (1966).
- Loy, R. & Moore, R. Y. *Expl. Neurol.* **57**, 645-650 (1977).
- Stenevi, U. & Björklund, A. *Neurosci. Lett.* **7**, 219-224 (1978).
- Crutcher, K. A. & Davis, J. N. *Brain Res.* **204**, 410-414 (1981).
- Cowan, N. M. *The Neurosciences, 4th Study Program* (eds Schmidt, F. O. & Woeden, F. G.) 59 (MIT Press, Cambridge, Massachusetts, 1979).
- McCaffery, C. A., Bennett, M. R. & Dreher, B. *Expl. Brain Res.* **48**, 377-386 (1982).
- Ebendal, T., Olson, L., Seiger, Å. & Hedlund, K. O. *Nature* **286**, 25-28 (1980).
- Vijayashankar, N. & Brody, H. *J. Neuropath. exp. Neurol.* **38**, 490-497 (1979).
- Whitehouse, P. J. *et al. Science* **215**, 1237-1239 (1982).
- Bondareff, W., Mountjoy, C. Q. & Roth, M. *Neurology (NY)* **32**, 164-168 (1982).
- Pearson, R. C. A., Gatter, K. G. & Powell, T. P. S. *Brain Res.* **261**, 321-325 (1983).
- Schmidt, R. H. *et al. J. Neurochem.* **38**, 738-748 (1982).
- Da Prada, M. & Zürcher, G. *Life Sci.* **19**, 1161-1174 (1976).

γ -Aminobutyric acid and glycine activate Cl^- channels having different characteristics in CNS neurones

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We have studied the properties of Cl^- channels in Müller cells of the lamprey brain, which are activated by two putative neurotransmitters, glycine and γ -aminobutyric acid (GABA). Previous studies^{1,2} have shown that in these cells the two ligands act through different receptors, and it was of interest to determine whether or not the two receptors, in turn, activate separate channels having different properties. We have shown previously^{3,4} that the channels activated by glycine have large conductances which decrease rapidly as the intracellular Cl^- concentration $[\text{Cl}^-]_i$ is increased above normal. We show here that GABA-activated channels have much smaller conductances which are independent of $[\text{Cl}^-]_i$ over the experimental range and also that the response to a saturating application of glycine is not occluded by simultaneous GABA application. These observations indicate that the glycine and GABA receptors are coupled to separate Cl^- channels having different characteristics.

Records were made from Müller cells in brains from adult lampreys (*Lampetra lamottenii*) using two intracellular electrodes, one for measuring transmembrane potential, the other for passing current. Experimental methods and procedures for analysing membrane current noise are described in detail elsewhere⁴. GABA (0.2 M) and glycine (0.25 M) were applied to the cells by pressure pulses to pipettes placed near the base of the dendrites. When drugs were applied simultaneously, the two pipette tips were adjusted to be less than 10 μm apart. Experiments were done at $5 \pm 1^\circ\text{C}$.

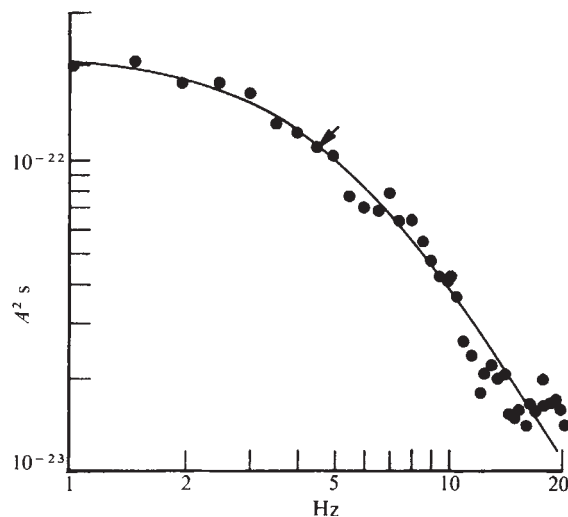


Fig. 1 Power density ($\text{A}^2 \text{s}$) as a function of frequency (Hz) for membrane current noise produced by application of GABA to a Müller cell by pressure pulses from a micropipette. Points are the means of 24 spectra smoothed by weighted averaging of adjacent points in groups of five. The smooth curve is of the form $S(f) = S(0)[1 + (f/f_c)^2]^{-1}$, where $S(f)$ is the power density and f the frequency; it was fitted to the results by eye by selection of the asymptotic power density, $S(0)$ and corner frequency, f_c . Reversal potential for the response (V_r) was -67 mV , holding potential (V_h) -57 mV , and mean current (I) 6.6 nA . Curve fit with $S(0) = 2.2 \times 10^{-22} \text{ A}^2 \text{s}$, $f_c = 4.5 \text{ Hz}$. Mean channel open time, $\tau = (2\pi f_c)^{-1} = 35.4 \text{ ms}$; channel conductance, $\gamma = S(0)/4\pi I(V_h - V_r) = 23 \text{ pS}$. Experiment done at 6°C .

An example of the power spectrum of GABA-induced current fluctuations with normal $[\text{Cl}^-]_i$ is shown in Fig. 1. The spectral distribution was consistent with a single-channel conductance of 23 pS and a mean open time of 35 ms. In eight experiments of this type, the mean channel conductance and open time ($\pm \text{s.d.}$) were $17 \pm 4 \text{ pS}$ and $42 \pm 4 \text{ ms}$, respectively. The conductance is much smaller than that reported previously⁴ for the response to glycine in these cells in comparable conditions, and

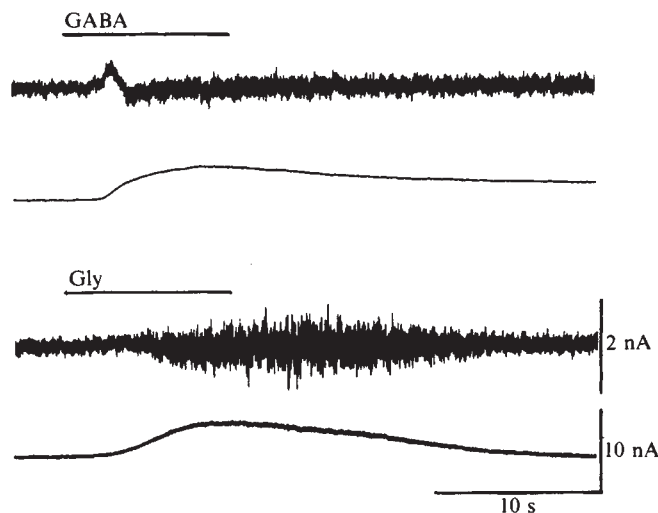


Fig. 2 Examples of current noise produced by GABA and glycine in the same Müller cell. Reversal potential for both responses was -65 mV, holding potential -55 mV. Both agonist applications produced maximum outward currents of ~ 4 nA (lower traces) but the increase in baseline noise due to GABA was much smaller than that due to glycine, indicating a much lower single-channel conductance. Calculated channel conductances and mean open times were, respectively, 14 pS and 45 ms for GABA and 63 pS and 32 ms for glycine.

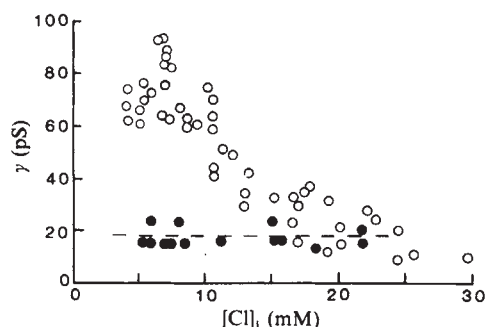


Fig. 3 ●, Conductance of GABA-activated channels plotted against intracellular Cl^- concentration. The slope of the regression line (0.008) is not significantly different from zero. ○, Results obtained previously⁴ for glycine-activated channels, shown for comparison. The behaviour of the two types of channel is markedly different. The intracellular Cl^- concentrations were calculated from the reversal potential for the responses⁴, using the Nernst equation.

Fig. 4 a, Effects on input resistance of maximal applications of GABA and glycine to a Müller cell. Cell resting potential was -67 mV and 8 -nA current pulses produced 10 -mV electrotonic potentials (downward deflections), indicating an input resistance of 1.25 M Ω . Glycine application (top trace) reduced the input resistance to 0.26 M Ω . Application of GABA from a second pipette produced a smaller reduction in input resistance, to 0.49 M Ω (middle trace). In the bottom trace, the effects of combined application of GABA and glycine summed to reduce input resistance to a minimum of 0.15 M Ω . If glycine and GABA receptors shared individual channels, occlusion rather than summation would have been expected with combined application (see text).

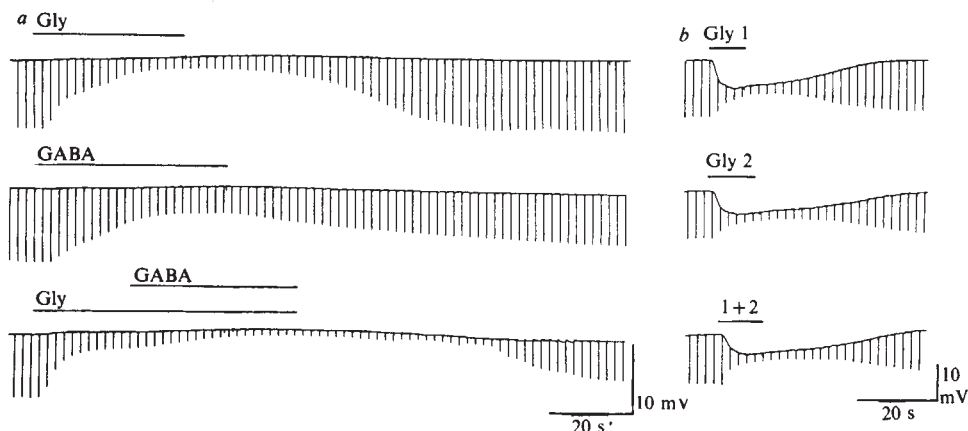


Fig. 4 b, When glycine was applied from both pipettes there was no such summation. In a cell with a resting potential of -57 mV, 8 -nA current pulses produced 15 -mV electrotonic potentials (input resistance 1.9 M Ω). Glycine application from the first pipette (Gly 1) reduced the input resistance to 0.26 M Ω ; application from the second pipette (Gly 2) and combined application ($1+2$) reduced the input resistance to 0.27 M Ω and 0.26 M Ω , respectively.

the open time is longer. The difference in conductance is apparent when the increase in baseline variance produced by the two agonists is compared in the same cell; see Fig. 2. The mean currents produced by GABA and glycine were similar but the increase in baseline variance produced by GABA was much smaller, indicating a much lower single-channel conductance.

To assess the effects of increased $[\text{Cl}^-]_i$ on the GABA-activated channels, cells were loaded with Cl^- by injection from KCl-filled pipettes. Mean channel conductance (18 ± 3 pS) and open time (43 ± 6 ms) were unchanged by Cl^- loading. Intracellular Cl^- concentrations ranged overall from 5 mM to 22 mM, calculated from the reversal potential for the response⁴. When channel conductance was plotted against $[\text{Cl}^-]_i$ (Fig. 3, solid circles), neither the slope of the resulting regression line (0.008) nor the correlation coefficient (0.011) was significantly different from zero. The overall conductance and mean open time were, respectively, 18 ± 4 pS and 42 ± 5 ms. These results are in marked contrast to those obtained previously with glycine-activated channels^{3,4}, also shown in Fig. 3 (open circles) for comparison.

The marked differences in single-channel conductance and effect of intracellular Cl^- on conductance suggest that GABA and glycine activate different populations of channels. Alternatively, the channels may be the same but have different conductance states when activated by the two agonists. Thus, GABA might open such a channel to its lowest conductance state and glycine to a higher conductance, depending on the intracellular Cl^- concentration. In support of this idea, it has been proposed⁵ that in cultured spinal neurones the conductance of GABA-activated channels is a sub-state of the glycine-activated conductance. Whether the channels in the Müller cells activated by the two agonists are the same or different cannot be decided with the techniques at hand. However, evidence can be obtained about whether the individual channels are shared by the glycine and GABA receptors. If they are, and if binding of the ligands to the receptors is reversible, then with normal $[\text{Cl}^-]_i$ the conductance change produced in a cell in response to a saturating application of glycine should be occluded by simultaneous application of GABA. In other words, in the presence of GABA, channels which would otherwise be activated by glycine would be opened to a lower conductance state and the overall macroscopic conductance change would be reduced. If, on the other hand, the two receptors activate separate channels, the conductances should sum. Summation was evident in four experiments similar to that shown in Fig. 4a. When the two pipettes were filled with glycine (Fig. 4b) no such summation occurred, indicating that the summation observed with GABA and glycine was due neither to lack of receptor saturation nor to spread of ligand from the second pipette outside the region activated by the first.

The conductance of the GABA-activated channels is in the range reported previously for other cells⁶⁻⁸; the mean open time is also consistent with previous studies when allowance is made for differences in temperature. Our results show that the Cl⁻ channels activated by GABA are quite different in their behaviour from those activated by glycine in the same cells. Furthermore, the lack of occlusion of the glycine response by simultaneous GABA application suggests that the glycine and GABA receptors do not share individual channels. This is in contrast to the previous suggestion⁹ that channels in cultured spinal neurones activated by GABA and glycine are shared. Whether the channels that are activated by GABA and glycine in Müller cells are intrinsically different or whether their different characteristics are agonist-induced, as proposed for cultured spinal neurones⁵, remains to be determined.

This work was supported by NIH research grant NS-09660.

Received 9 November 1983; accepted 24 January 1984.

1. Martin, R. J. *Comp. Biochem. Physiol.* **61C**, 37-40 (1978).
2. Matthews, G. & Wickelgren, W. O. *J. Physiol., Lond.* **293**, 393-415 (1979).
3. Gold, M. R. & Martin, A. R. *Nature* **299**, 828-830 (1982).
4. Gold, M. R. & Martin, A. R. *J. Physiol., Lond.* **342**, 99-117 (1983).
5. Hamill, O. P., Bormann, J. & Sackmann, B. *Nature* **305**, 805-808 (1983).
6. Dudel, J., Finger, W. & Stettmeier, H. *Pflügers Arch. ges. Physiol.* **387**, 143-151 (1980).
7. Cull-Candy, S. G. & Miledi, R. *Proc. R. Soc. B211*, 527-535 (1981).
8. Barker, J. L., McBurney, R. N. & MacDonald, J. F. *J. Physiol., Lond.* **322**, 365-387 (1982).
9. Barker, J. L. & McBurney, R. N. *Nature* **277**, 234-236 (1979).

Serum β_2 -microglobulin binds to a T-cell differentiation antigen and increases its expression

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The human T-cell leukaemia and differentiation antigen HTA 1 is defined by the monoclonal antibody NA1/34 (ref. 1) and also recognized by the monoclonal antibody OKT6². Like class I products of the human major histocompatibility complex, it has a glycosylated heavy (α) chain of approximately 45-50,000 molecular weight (MW) in non-covalent association with β_2 -microglobulin (β_2m) (MW 11,900). A particular feature of HTA 1 is the presence in significant amounts of an additional β_2m -like subunit, called β_t (refs 3, 4). To facilitate biochemical studies we have prepared a high HTA 1 expressor variant (NH17) of the human thymoma line MOLT-4⁵. The N-terminal amino acid sequence of the β_t purified from this cell line was shown to be indistinguishable from that of bovine β_2m . Further, β_t was present when the cells were grown in medium containing fetal calf serum (FCS), but absent from cells grown with human serum (HuS). We show here that addition of human and bovine β_2m to MOLT-4 and NH17 cells grown in serum-free medium produces a significant elevation of HTA 1 antigen expression, providing evidence for a regulatory or stabilizing function for the exchange of extracellular β_2m with a cell-surface antigen.

HTA 1 was purified from detergent lysates of bulk preparations of NH17 cells by affinity chromatography with NA1/34 (ref. 6). The three HTA 1 components were sequentially eluted from the NA1/34 affinity column at 40 °C with a pH gradient in the order β_2m (pH 8.5-9.5), β_t (pH 9.5-10.7) and α (pH 10.5-11.6) (Fig. 1a), and fractions containing pure β_2m and β_t were taken for N-terminal amino acid sequence determination. The N-terminal sequence of β_t was indistinguishable from that of bovine β_2m while differing from that of human β_2m at positions 4, 15, 17, 20 and 22 (Fig. 2). The other light-chain

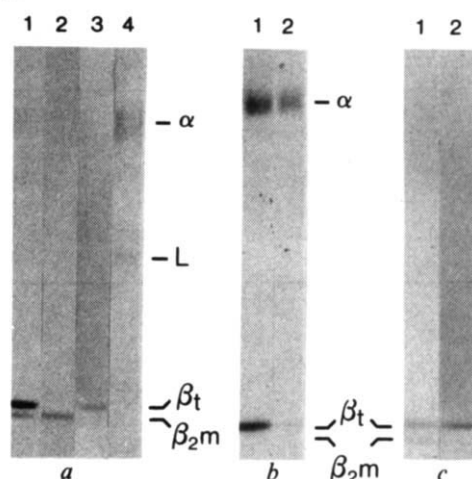


Fig. 1 Analysis of HTA 1 components by SDS-PAGE in non-reducing conditions. *a*, Coomassie blue-stained gel showing HTA 1 purified from NP-40 lysates of NH17 cell membranes by immunoabsorption on NA1/34 Sepharose as previously described⁶ except that the column was washed at 4 °C with 10 bed-volumes of 50 mM Tris, pH 8.0, 0.5 M NaCl, 5 mM EDTA, 0.5% NP-40, and five bed-volumes of 10 mM Tris, pH 7.4, and eluted (lane 1) at 4 °C with 50 mM diethylamine, or (lanes 2-4) at 40 °C with 50 mM diethylamine, adjusted with redistilled 1 M N-ethylmorpholine acetate to give a pH gradient at room temperature from pH 7.5 to 11.6. The elution profile is shown at pH 8.0 (lane 2), pH 10.2 (lane 3) and pH 11.6 (lane 4). A parallel gel in reducing conditions, under which the α chain is more clearly seen, confirmed the presence of the α chain in lanes 1 and 4 and its absence in lanes 2 and 3. Band 'L' has been identified by N-terminal sequence as immunoglobulin light chain which leaches from the affinity column in the hot alkaline conditions. *b*, Surface ¹²⁵I-labelled HTA 1 purified with NA1/34 was prepared as described¹³ from NH17 cells grown continuously in the presence of 5% fetal calf serum (lane 1), or (lane 2) 5% normal AB Rh⁺ human serum. *c*, Coomassie blue - stained gel showing β_t/β_2m marker (lane 1), and bovine β_2m purified from fetal calf serum (lane 2). Fetal calf serum (750 ml) was passed over a 5 ml column of BBM.1 Sepharose, the column washed as described above, and the bound material eluted with 50 mM diethylamine and immediately neutralized with redistilled N-ethylmorpholine acetate. (The monoclonal antibody BBM.1⁷ was the gift of W. F. Bodmer.) SDS-PAGE was performed on 9-16% linear acrylamide gradients as described previously⁶.

component of HTA 1 was confirmed to be human β_2m by comparison with the published sequence (Fig. 2).

The serum origin of β_t was confirmed by immunoprecipitation of surface-radioiodinated membrane proteins from NH17 cells grown in medium supplemented with either bovine or human serum. β_t is present as a strongly iodinated band in NA1/34 precipitates from cells grown in medium containing FCS but is absent from cells grown in medium containing only HuS (Fig. 1b). The HTA 1 from cells exposed only to HuS contains a single light chain which comigrates with human β_2m . Furthermore, when bovine β_2m was purified from FCS with the anti-human β_2m monoclonal antibody, BBM.1 (ref. 7) the major band in the eluate co-migrated on non-reducing SDS-polyacrylamide gel electrophoresis (PAGE) with β_t (Fig. 1c).

The binding of exogenous β_2m to HTA 1 may represent exchange for endogenous β_2m in a manner analogous to the observed exchange of the β_2m component of human HLA and mouse H-2 with homologous and heterologous β_2m (refs 8, 9). However, we cannot exclude the possibility that the HTA 1 α chain can associate with more than one β_2m molecule, or that a proportion of HTA 1 α chain exists on the cell surface unassociated with β_2m but still able to bind it. The molar relationship of the components of the HTA 1 complex is misrepresented in radioiodinated cell-surface preparations since β_t is iodinated to a specific activity some 100-fold higher than that of β_2m (ref. 6). The true stoichiometry of the HTA 1 molecule is also difficult

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Table 1 Effect of additives to serum-free medium on the expression of HTA-1 and HLA-A, B, C.

Additives to medium (20 h)	Monoclonal antibody			
	NA1/34 (HTA 1)		W6/32 (HLA-A,B,C)	
	Cells		Cells	
	NH17	MOLT-4	NH17	MOLT-4
	Peak fluorescence			
None	488	100	95	100
5% FCS	648	106	94	107
Bovine β_2m (10 $\mu g\ ml^{-1}$)	904	129	101	105
5% human serum	512	82	93	100
Human β_2m (10 $\mu g\ ml^{-1}$)	808	139	97	97

Cells (MOLT-4 and the high HTA 1 expressor clone NH17B.5.5) were grown in serum-free Iscove's medium (Flow Labs), supplemented with 3.75 g l⁻¹ NaHCO₃, 0.11 g l⁻¹ Na pyruvate, 0.584 g l⁻¹ glutamine and 5 mg ml⁻¹ insulin, for at least 1 week before the experiment. In these conditions, providing the cell density was kept at about 5 × 10⁵ cells ml⁻¹ or more, growth was not visibly impaired. After incubation for 20 h in the presence of the additives the binding of the monoclonal antibodies was measured with a FACS II cytofluorimeter using FITC-sheep anti-mouse Ig as second antibody as described previously¹³, except that fetal calf serum was omitted. (Bovine β_2m was from A. Sanderson and human β_2m from Sigma.) The numbers represent the peak position of the fluorescent profile relative to MOLT-4 and arbitrarily taken for each monoclonal antibody as 100.

to determine in unlabelled bulk preparations purified on NA1/34 immunoadsorbents. The α -chain binds tightly to the antibody and elution may not be quantitative. Furthermore, the light chain components are weakly associated with the heavy chain at room temperature and 37 °C³ and may therefore be underestimated. In fact, human β_2m is more easily dissociated from HTA 1 than the bovine molecule (Fig. 1a) (see also ref. 10), and also may be completely removed from the complex, without removing β_1 , by prior immunoabsorption with BBM.1 at 4 °C (data not shown). This weak interaction contrasts with interactions between other polypeptide chains which tend to be strong even in the absence of interchain disulphide bonds. Apart from β_2m there is little biological precedent for exchange reactions involving the subunits of multimeric proteins in physiological conditions.

The lability of the isolated HTA 1 complex, the capacity of cellular HTA 1 to bind to exogenous β_2m and the proposed conformational stability conferred on class I heavy chains by β_2m (ref. 11), suggested that extracellular β_2m may modulate the stability or expression of HTA 1 at the cell surface. To test this, MOLT-4 and NH17 cells were first adapted to growth in serum-free medium. HTA 1 expression in these cells could be increased 1.5–2-fold by incubation with 10 $\mu g\ ml^{-1}$ human or bovine β_2m (Table 1) for 20h. Somewhat lower effects were seen after addition to the growth medium of 5% FCS, while 5% HuS had no significant effect. This may reflect the difference in β_2m concentration between normal human serum (1–2 $\mu g\ ml^{-1}$) and fetal serum, which may be some fivefold higher¹². In the absence of added β_2m the level of HTA 1 decreases markedly at low cell density, probably reflecting the exogenous concentration of β_2m secreted by the cells (data not shown).

These additions had no effect on the expression of HLA (Table 1). β_2m secretion by the cells themselves, although inadequate for the full expression of HTA 1, may be sufficient to sustain the β_2m -HLA exchange reaction in the direction which favours the complex. We have previously reported the presence of small amounts of β_1 in HLA and the correlation of this with HTA 1 expression⁶. We now believe that the high amount of HTA 1 on NH cells acts to concentrate exogenous β_2m at the cell surface, possibly favouring exchange to neighbouring membrane molecules.

Since HTA 1 expression on cultured cells may be modified by varying the β_2m concentration in the medium, it is feasible

	5	10	15	20
HTA-1 β_t	- Q R P P K / F I Q V Y S R H P P E D G K / F P N Y L N			
Bovine β_2m	I Q R P P K I Q V Y S R H P P E D G K P N Y L N			
HTA-1 β_2m	- Q R T P K / F I Q V Y S R H P A E N G K / F S N K / F L N			
Human β_2m ¹⁴	I Q R T P K I Q V Y S R H P A E N G K S N F L N			

Fig. 2 N-terminal amino acid sequences of β_2m and β_t purified from NH17 and related proteins. Approximately 0.6 nmol of the light-chain components of HTA 1 were purified as described in Fig. 1 and the N-terminal sequence determined by automated solid phase microsequencing on an instrument designed and built in the laboratory^{15,16}. This method does not identify the initial N-terminal amino acid. Lysine (K) and phenylalanine (F) residues co-chromatographed in these analyses. Bovine β_2m was purified as described elsewhere¹⁷ (gift of A. Sanderson) and was N-terminally sequenced both by the microsequencing technique described above and separately in a modified spinning cup sequencer¹⁸. This sequence differs from the published sequence¹⁷ which gives asparagine (N) at position 17.

that the phenotype of differentiating T cells is influenced similarly in the thymic microenvironment by levels of exogenous β_2m . Thus, β_2m , which is present in all body fluids and is elevated in the serum in various disease states¹², may thereby influence intercellular recognition events during T-cell ontogeny.

We thank John Walker for assistance with N-terminal microsequencing, David Gilmore for flow cytometry and John Jarvis and Richard Pannell for technical assistance. R.K. is supported by a Florey Fellowship of the Royal Society of London and a Buck Memorial Studentship from St John's College, Cambridge. F.C. is supported by an EMBO fellowship.

Received 23 November 1983; accepted 17 January 1984.

- McMichael, A. J. *et al.* *Eur. J. Immun.* **9**, 205–210 (1979).
- Cotner, J., Mashimo, H., Kung, P. C., Goldstein, G. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **78**, 3858–3862 (1981).
- Ziegler, A. & Milstein, C. *Nature* **279**, 243–244 (1979).
- van Agthoven, A. & Terhorst, C. *J. Immun.* **128**, 426–432 (1982).
- Burrone, O. R., Calabi, F., Kefford, R. F. & Milstein, C. *EMBO J.* **2**, 1591–1595 (1983).
- Calabi, F., Burrone, O. R. & Milstein, C. *Molec. Biol. Med.* **1**, 219–223 (1983).
- Brodsky, F. M., Bodmer, W. F. & Parham, P. *Eur. J. Immun.* **9**, 536–545 (1979).
- Hyafil, F. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5834–5838 (1979).
- Schmidt, W., Festenstein, H., Ward, P. J. & Sanderson, A. R. *Immunogenetics* **13**, 483–491 (1981).
- Sanderson, A. R. & Ward, P. J. *Immunology* **83**, 87–92 (1983).
- Lancet, D., Parham, P. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3844–3848 (1979).
- Poulik, M. D. & Reisfeld, R. A. in *Contemporary Topics in Molecular Immunology* Vol 4 (eds. Inman, F. P. & Mandy, W. J.) 157–204 (Plenum, New York, 1975).
- Burrone, O. R. & Milstein, C. *EMBO J.* **1**, 345–349 (1982).
- Cunningham, B. A., Wang, J. L., Berggard, I. & Peterson, P. A. *Biochemistry* **12**, 4811–4822 (1973).
- Walker, J. E. *et al.* *Eur. J. Biochem.* **123**, 253–260 (1982).
- Walker, J. E. & Gay, N. J. *Meth. Enzym.* **97**, 195–218 (1983).
- Groves, M. L. & Greenberg, R. *J. Biol. Chem.* **257**, 2619–2626 (1982).
- Runswick, M. J. & Walker, J. E. *J. Biol. Chem.* **258**, 3081–3085 (1983).

β_2 -Microglobulin from serum associates with MHC class I antigens on the surface of cultured cells

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β_2 -Microglobulin (β_2m) is a highly conserved polypeptide (12,000 molecular weight; 12K) noncovalently associated with the heavy chain (45–48K) of the major histocompatibility complex (MHC) class I antigens. Its synthesis is required for expression of the HLA-A/B and H-2K/D heavy chains at the cell surface¹; β_2m is also associated with the human cell-surface antigens T6 and M241 isolated from thymocytes^{2–6}. However, on the T leukaemic cell line MOLT-4 some of the T6 antigens

contain a different 12K subunit, termed β_1 (refs 3, 7, 8). Purified human β_2 -m can exchange partially both with human β_2 -m associated with HLA-antigens⁹, and with mouse β_2 -m associated with murine alloantigens^{10,11}. As MOLT-4 cells were grown in the presence of fetal calf serum (FCS) and as serum is known to contain some free β_2 -m¹², we examined whether β_1 was bovine β_2 -m which had replaced endogenous β_2 -m on the surface of the cell. Here we show both that β_2 -m from FCS or human serum (HuS) used in cell culture can exchange with β_2 -m on the cell surface, and that β_1 is in fact bovine β_2 -m.

MOLT-4 cells cultured in RPMI 1640 medium supplemented with either 10% FCS or 0.2 $\mu\text{g ml}^{-1}$ bovine β_2 -m could bind a rabbit anti-bovine β_2 -m serum (KF59) as judged by indirect

immunofluorescence, whereas cells cultured in human serum could not (data not shown). To analyse this in more detail, MOLT-4 cells were radiolabelled with Na^{125}I and immunoprecipitates made with KF59 were subjected to SDS-polyacrylamide gel electrophoresis (PAGE). As shown in Fig. 1a (lanes 1, 2), bovine β_2 -m could be isolated only from cells cultured in FCS (but not HuS). Bovine β_2 -m was associated mainly with a 49K protein (lane 2), which was identified as T6. The T6 antigen isolated from MOLT-4 cells, which had been cultured in the presence of FCS, contained a 12K subunit which comigrated with bovine β_2 -m (Fig. 1a, lanes 3, 8, 9) on SDS-PAGE. In contrast, the T6 heavy chain isolated from MOLT-4 cells which had been cultured in a medium containing HuS

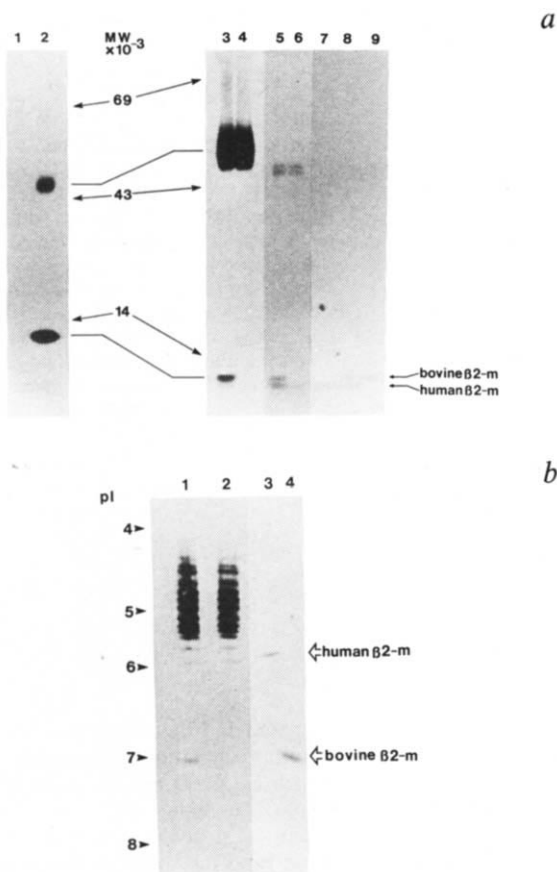


Fig. 1 Analysis of T6 and HLA-A/B antigens by SDS-PAGE and isoelectric focusing. *a*, Analysis on SDS-PAGE using the Tris/Bicine system with 3 M urea⁷. Lanes 1-6 represent autoradiograms², lanes 7-9 are from another part of the same gel stained with Coomassie brilliant blue. Immunoprecipitates were made from MOLT-4 cells cultured in the presence of FCS (lanes 2, 3, 5) or HuS (lanes 1, 4, 6). Lanes 1, 2, rabbit anti-bovine β_2 -m (KF59); lanes 3, 4, anti-T6 (IIC7); lanes 5, 6, anti-HLA-A/B (W6/32); lane 7, human β_2 -m; lane 8, human β_2 -m and bovine β_2 -m; lane 9, bovine β_2 -m. *b*, Analysis by isoelectric focusing³ of ^{125}I -labelled T6 antigens from MOLT-4 cells cultured in the presence of FCS (lane 1) or HuS (lane 2). Lanes 1 and 2 represent an autoradiogram and lane 3 (human β_2 -m) and lane 4 (bovine β_2 -m) are from the other half of the same isoelectric focusing gel stained with Coomassie brilliant blue.

Methods: MOLT-4 cells were cultured in RPMI 1640 medium (Gibco) supplemented with either 10% FCS or HuS. After surface radioiodination cells were lysed in a buffer containing 1% Nonidet-P40 and debris was removed by centrifugation at 12,000g for 15 min⁶. Immunoprecipitations were carried out with a rabbit anti-bovine β_2 -m serum (KF59) (a gift from Dr K. Kithier) and monoclonal antibodies specific for human β_2 -m (A88)⁴, the T6 antigen (IIC7)⁴ or at HLA-A/B antigens (W6/32)¹³ as described elsewhere⁶. Human β_2 -m was purified according to Cejka and Kithier¹⁴ and bovine β_2 -m was a gift from Dr M. L. Groves (Food Science Laboratories, Philadelphia)¹⁵.

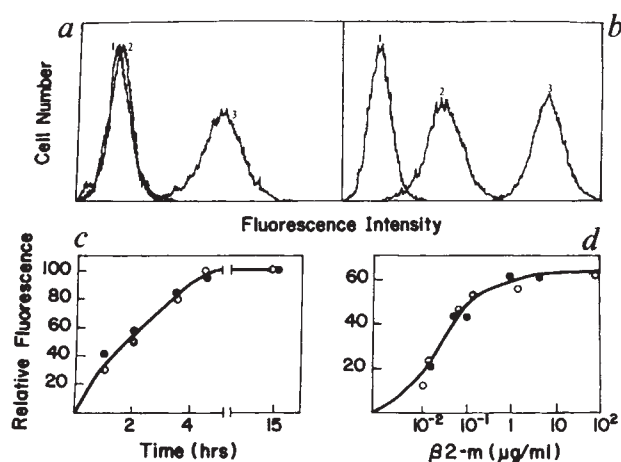


Fig. 2 Detection of bovine and human β_2 -m at the surface of murine L cells by indirect immunofluorescence. *a*, Detection of human β_2 -m. (1) L cells cultured in HuS and stained with normal mouse serum. (2) L cells cultured in FCS and stained with anti-human β_2 -m (A88). (3) L cells cultured in HuS and stained with A88. *b*, Detection of bovine β_2 -m. (1) L cells cultured in FCS and stained with normal rabbit serum. (2) L cells cultured in HuS and stained with rabbit anti-bovine β_2 -m (KF59). (3) L cells cultured in FCS and stained with KF59. L cells were cultured in Dulbecco's modified essential medium (DMEM, Gibco) supplemented with 10% FCS or 10% HuS. L cells were detached from the Petri dishes with 0.05% trypsin, washed in phosphate-buffered saline (PBS) and incubated with anti-bovine β_2 -m serum (1:50) or an anti-human β_2 -m monoclonal reagent (1:1,000) for 30 min at 4 °C¹⁶. The cells were washed twice with PBS and incubated with fluorescein isothiocyanate-coupled goat anti-rabbit-IgG (Kirkgaard & Perry) or with fluorescein isothiocyanate coupled goat anti-mouse Fab (Coulter) for 30 min on ice. After two washes with PBS fluorescence was analysed on an Epics V cytofluorograph using a logarithm display. *c*, Kinetic study of the binding of human β_2 -m or bovine β_2 -m from serum to mouse L cells. L cells (10×10^6) previously cultured in FCS were incubated with 10% HuS for different time periods at 37 °C in a humidified atmosphere with 5% CO_2 . Conversely, L cells previously cultured in HuS were incubated with FCS. Trypsin detached cells were then prepared for indirect immunofluorescence with a monoclonal antibody A88 specific for human β_2 -m (open circles) or rabbit anti-bovine β_2 -m (KF59) (closed circles). Fluorescence intensity was analysed on an Epics V using a linear scale. The fluorescence intensities are expressed as percentages of the fluorescence intensity obtained with cells cultured in HuS or FCS for at least 2 weeks. *d*, Binding of purified β_2 -m to L cells. L cells (10×10^6) were incubated with either purified human or bovine β_2 -m after they had been cultured with FCS or HuS, respectively, in RPMI 1640 medium supplemented with 5 $\mu\text{g ml}^{-1}$ insulin, 35 $\mu\text{g ml}^{-1}$ transferrin (Sigma) and varying concentrations of purified β_2 -m, at 37 °C. After 2 h, excess β_2 -m was removed by washing the cells three times with PBS. Cells were detached by trypsinization and analysed by indirect immunofluorescence with anti-bovine β_2 -m (●) or anti-human β_2 -m (○)¹⁶. The relative fluorescence intensity was determined from a linear display on an Epics V cell sorter and compared with the fluorescence intensity obtained with cells which had been cultured in FCS or HuS for at least 2 weeks (100% value).

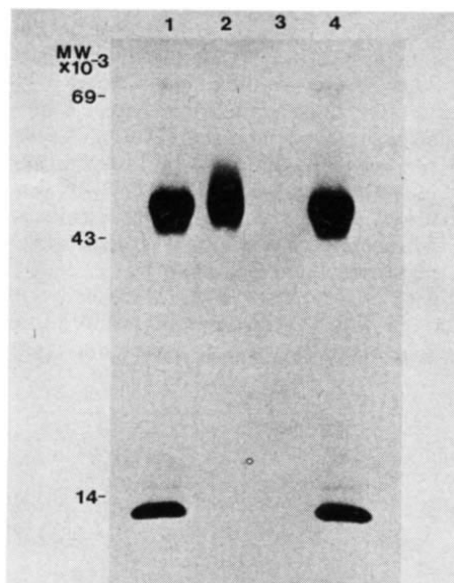


Fig. 3 Autoradiogram of SDS-PAGE analysis of H-2K/D antigens after exchange with human β_2 -m. L cells (20×10^6) which had been cultured in the presence of FCS were detached with trypsin and cell-surface radioiodinated as described elsewhere¹⁶. These cells were then incubated in DMEM containing 10% of either HuS (lanes 1, 2) or FCS (lanes 3, 4) for 2 h at 37 °C in a 5% CO₂ atmosphere and before lysis. The lysates were immunoprecipitated with the monoclonal antibody M1/42.3 directed against all H-2K/D antigens¹⁷ (gift from Dr T. Springer) (lanes 1 and 4) and with the monoclonal antibody A88 specific for human β_2 -m (lanes 2, 3). SDS-PAGE was carried out on discontinuous vertical slab gels (10–15% acrylamide gradient) according to a modification of Laemli procedure¹⁸.

contained a band in the position of human β_2 -m which showed up after a longer exposure (Fig. 1a, lane 4). The HLA-A/B antigens isolated from MOLT-4 cells cultured in the presence of FCS contained bands which co-migrated with both human and bovine β_2 -m (Fig. 1a, lanes 5, 6). Further proof that T6 associated with bovine β_2 -m was provided by isoelectric focusing analysis (Fig. 1b). Cells grown in a medium with FCS expressed a T6 antigen which contained a band (*pI* 7.0), co-migrating with bovine β_2 -m (Fig. 1b, lane 1). When MOLT-4 cells were cultured in the presence of HuS, this band was absent (Fig. 1b, lane 2). Interestingly, bovine β_2 -m was never found in association with the M241 antigen, which has structural homology with T6⁶ (data not shown). Taken together these results demonstrate that the so-called β_1 (refs 7, 8) is in fact bovine β_2 -m and that bovine β_2 -m can associate selectively with some human MHC class I antigen heavy chains.

For a more detailed study of possible exchange between serum and native β_2 -m, murine L cells were used. As shown in Fig. 2a, b, human and bovine β_2 -m could be detected by indirect immunofluorescence at the cell surface of L cells cultured for 4 h in the presence of either HuS or FCS. Reactivity of L cells cultured in HuS with the antiserum KF59 is probably due to some cross-reactivity among β_2 -m (Fig. 2b). Samples taken at different times revealed that the binding of bovine or human β_2 -m from serum could be detected after 30 min and reached a plateau at 5 h (Fig. 2c). Binding of purified bovine or human β_2 -m occurred at low concentrations of β_2 -m in the medium ($0.01 \mu\text{g ml}^{-1}$) and a plateau was reached at $0.5 \mu\text{g ml}^{-1}$ (Fig. 2c). This value is close to the concentration of β_2 -m in the culture medium (10% serum), if a serum concentration of $1\text{--}2 \mu\text{g ml}^{-1}$ (ref. 12) is assumed.

To show that an exchange occurred rather than just binding of serum β_2 -m to MHC class I antigens, the following experiments were carried out. First, L cells were cultured in the

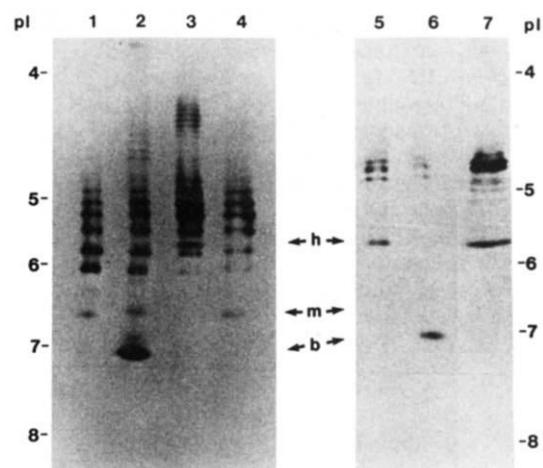


Fig. 4 Analysis by isoelectric focusing of several class I MHC antigens from L cells. L cells or L cells expressing HLA-B7¹⁶ (CM7.13) were cultured in DMEM supplemented with 10% FCS or HuS. JY cells were cultured in RPMI 1640 medium with FCS. After cell surface radioiodination cells were lysed in Nonidet-P40 and lysates were used for immunoprecipitations¹⁶. Immunoprecipitates were made with monoclonal anti-H-2K/D (M1/42.3), monoclonal anti-human β_2 -m (A88), rabbit anti-bovine β_2 -m (KF59) or with a monoclonal anti-HLA-B antigens (4E) (a gift from Dr Bo Dupont). An autoradiogram of the isoelectric focusing gel was prepared as described elsewhere¹⁶. The position of human (h), bovine (b) and mouse (m) β_2 -m is indicated by arrows.

Lane	Cell	Serum in culture	Antibody
1	L cells	FCS	M1/42.3
2	L cells	FCS	KF59
3	L cells	HuS	A88
4	L cells	HuS	M1/42.3
5	CM7.13	HuS	4E
6	CM7.13	FCS	4E
7	JY	FCS	4E

presence of FCS, radioiodinated and then incubated for 2 h in the presence of HuS. Immunoprecipitations demonstrated that the ¹²⁵I- β_2 -m associated with H-2K/D antigens was replaced by unlabelled human β_2 -m. The monoclonal antibody A88 which is specific for human β_2 -m could precipitate the H-2K^k/D^k heavy chains, but no band was found in the position of β_2 -m (Fig. 3, lane 2). Second, analysis by isoelectric focusing showed that immunoprecipitates made with KF59 (anti-bovine β_2 -m) or A88 (anti-human β_2 -m) contained only the corresponding exogenous β_2 -m, but not murine β_2 -m (Fig. 4, lanes 2, 3). These results demonstrate that β_2 -m bound to class I antigens is indeed replaced on the cell surface by exogenous β_2 -m.

The H-2K^k/D^k antigens immunoprecipitated with a monoclonal antibody (M1/42.3) directed at all H2-K/D antigens¹⁷ contained only murine β_2 -m (Fig. 4, lanes 1, 4). Since the H2-K^k/D^k antigens could also be precipitated with antibodies against bovine and human β_2 -m, (Fig. 4, lanes 2, 3) this result indicated that the H-2K^k/D^k antigens were not recognized by the M1/42.3 antibody when associated with exogenous β_2 -m. This conclusion is supported by the finding that immunoprecipitates made with M1/42.3 from L cells after exchange with ¹²⁵I-labelled human β_2 -m did not contain any radioactivity (data not shown).

Earlier, we and others had shown that the gene coding for the human HLA-B7 heavy chain could be expressed in L cells^{16,19}. When L cells (clone CM7.13) expressing HLA-B7 (ref. 16) were cultured in the presence of HuS, the HLA-B7 antigen was completely associated with human β_2 -m (Fig. 4, lane 5). Culturing in the presence of FCS resulted in HLA-B7 heavy chain which was completely associated with bovine β_2 -m

(Fig. 4, lane 6). Fig. 4 (lane 7) also shows that the HLA-B7 antigen from the human lymphoblastoid cell line JY cultured in the presence of FCS contains only endogenous human β_2 -m. This suggests that the serum β_2 -m may replace the murine β_2 -m associated with HLA-B7 heavy chain more rapidly than the human β_2 -m associated with HLA-B7 heavy chains. Indirect immunofluorescence studies indicated that in general the extent of exchange on L cells (see Fig. 2) was greater than on several human cells (MOLT-4, HPB-ALL and thymocytes) (data not shown).

In an attempt to quantitate the exchange reactions in more detail, we compared the radioactivity in endogenous and exogenous β_2 -m after separation by electrophoresis. On MOLT-4 cells, equal amounts of radioactivity were found in both human and bovine β_2 -m associated with HLA-A/B antigens. Since the specific activity of ^{125}I -labelled β_2 -m's may vary drastically², this could be an overestimate. A more accurate estimate of the extent of exchange was made on L cells. Antibody M1/42.3 was used to measure the amount of radioactivity in the H-2K^k/D^k antigens associated with murine β_2 -m. Conversely, the antibodies directed at human or bovine β_2 -m were used to measure the amount of ^{125}I in the H-2K^k/D^k antigens bound to exogenous β_2 -m. We determined approximately 80% exchange on L cells.

This β_2 -m exchange phenomenon may have many implications for *in vitro* studies of the MHC class I antigens. For instance, β_2 -m replacement may be the cause of the so-called 'serum dependent cytotoxicity'²¹⁻²⁴. It may also complicate the interpretation of MHC class I gene transfer experiments, when the level of murine β_2 -m expression is used for detection of gene products²⁵. Conversely, the β_2 -m exchange could be used advantageously in other experiments. For instance, culturing of L cells in human serum after transfer of human MHC class I genes may increase the possibility of precise serological identification or recognition by cytotoxic T lymphocytes¹⁶. The presence of β_2 -m in serum and the β_2 -m exchange phenomenon may also be of importance for the regulation of the expression of some MHC class I genes *in vivo*.

We thank Dr M. L. Groves for the purified bovine β_2 -m, Drs B. Dupont, K. Kithier and T. Springer for antibody reagents and Drs J. Michaelson and M. Mescher for their critical reading of the manuscript. This work was supported by grants from the N.I.H. (AI-15066), American Cancer Society (IM-289) and March of Dimes (1-832). C.T. is a scholar of the Leukemia Society of America. C.B. is a recipient of a fellowship from the United States-Spanish Joint Committee for Scientific and Technological Cooperation.

Received 10 October; accepted 19 December 1983.

- Ploegh, H. L., Orr, H. T. & Strominger, J. L. *Cell* **24**, 287-299 (1981).
- Terhorst, C. *et al.* *Cell* **23**, 771-780 (1981).
- van Agthoven, A. & Terhorst, C. *J. Immunol.* **128**, 426-432 (1982).
- Lerch, P. G., van de Rijn, M., Schrier, P. & Terhorst, C. *Hum. Immunol.* **6**, 13-30 (1983).
- Knowles, R. W. & Bodmer, W. F. *Eur. J. Immunol.* **12**, 676 (1982).
- van de Rijn, M., Lerch, P., Knowles, R. & Terhorst, C. *J. Immunol.* **131**, 851-855 (1983).
- Ziegler, A. & Milstein, C. *Nature* **279**, 243-244 (1979).
- Burroni, O. R., Calabi, F., Kefford, R. F. & Milstein, C. *EMBO J.* **2**, 1591-1595 (1983).
- Hyafil, F. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5834-5838 (1979).
- Schmidt, W., Festenstein, H., Ward, P. J. & Sanderson, A. R. *Immunogenetics* **13**, 483-491 (1981).
- Hester, R. B., Kubo, R. T. & Grey, H. M. *Scand. J. Immunol.* **9**, 125-134 (1979).
- Berggard, I. & Bearn, A. G. *J. biol. Chem.* **243**, 4095-4103 (1968).
- Barnstable, C. J. *et al.* *Cell* **14**, 9-20 (1978).
- Cejka, J. & Kithier, K. *Archs Biochem. Biophys.* **153**, 854-861 (1972).
- Groves, M. L. & Greenberg, R. *J. biol. Chem.* **257**, 2619-2626 (1982).
- Bernabeu, C. *et al.* *J. Immunol.* **131**, 2032-2037 (1983).
- Stallcup, K. C., Springer, T. A. & Mescher, M. F. *J. Immunol.* **127**, 923-927 (1981).
- Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- Barbosa, J. A. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **79**, 6327-6331 (1982).
- Terhorst, C. P., Parham, P., Mann, D. L. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 910-914 (1976).
- Shustik, C., Cohen, I. R., Schwartz, R. S., Latham-Griffin, E. & Waksal, S. D. *Nature* **263**, 699-701 (1976).
- Forni, G. & Green, I. *J. Immunol.* **116**, 1561-1565 (1976).
- Tsutsui, I. & Everett, N. B. *Cell. Immunol.* **10**, 359-370 (1974).
- Peck, A. B., Anderson, L. C. & Wigzell, M. *J. exp. Med.* **145**, 802-818 (1977).
- Goodenow, R. S. *et al.* *Nature* **300**, 231-235 (1982).

The nature of endothelium-derived vascular relaxant factor

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The existence of endothelium-derived vascular relaxant factor (EDRF) was postulated by Furchgott and colleagues¹ when they observed that acetylcholine paradoxically relaxed precontracted aortic strip preparations by an endothelium-dependent mechanism. This phenomenon has since been demonstrated in different blood vessels and mammalian species and it can be elicited by several other agents¹⁻⁶. EDRF has been thought to be a humoral agent, a lipoxigenase derivative and possibly a free radical⁷. In the study reported here, by using aortic preparations from the rabbit, alone and in cascade experiments with isolated perfused coronary preparations, we demonstrate definitively that EDRF is a humoral agent. It is released from unstimulated aortic preparations containing endothelium, its release can be stimulated for prolonged periods by acetylcholine, and it is not a lipoxigenase derivative or free radical but an unstable compound with a carbonyl group at or near its active site.

Rabbits (2.5 kg, New Zealand White) were stunned and 2 mm-wide ring segments from the descending thoracic aorta were prepared, mounted isometrically at 1.2 g resting force in a tissue bath with oxygenated (95% O₂, 5% CO₂) Holman's solution at 37 °C, and equilibrated for 90 min. The hearts were removed, the left coronary artery was cannulated, perfused and superfused with oxygenated Holman's solution pH 7.2-7.4, at 37 °C, then dissected free from the heart, distally cannulated, and its side branches tied; perfusion continued at constant flow (7-8 ml min⁻¹) to give a transmural pressure of 20 mm Hg (ref. 7). Tension and pressure were measured and recorded on a chart recorder. In cascade experiments, 7 cm of descending thoracic aorta was perfused intraluminally and the effluent pumped at the same flow rate into a coronary artery denuded of endothelium, with the facility to infuse drugs continuously before the aorta or into the intervening tubing with a microprocessor-controlled pump. Endothelium was removed from aortic strips by gentle abrasion with filter paper, from perfused aortae by reaming, and from perfused coronary arteries by 5-min perfusion with high K⁺ buffer (40 mM)⁷. It was confirmed as being present or absent histologically by *en face* silver staining in the appropriate experimental conditions. All reagents were of analytical grade.

Aortic strips, precontracted with 10⁻⁵ M 5-hydroxytryptamine (5-HT) were relaxed by acetylcholine (ACh) (Fig. 1a, b), or by the ionophore A23187 (Fig. 1c), in the presence of intact endothelium but not in its absence. This relaxation could be inhibited in a concentration-dependent manner by potassium borohydride (KBH₄), dithiothreitol, phenylhydrazine or phenidone (Fig. 1) and by certain other agents (see Table 1); these agents did not affect aortic preparations denuded of endothelium at the concentrations used (except where indicated in Table 1).

In cascade perfusion experiments, coronary artery preparations were denuded of endothelium (to enhance constrictor responses and avoid any complicating influence of endothelium), precontracted (to demonstrate dilator influences), and used to bioassay EDRF released from aortae. Stable coronary constriction was achieved by infusing into the intervening tubing 5-HT plus ACh to final concentrations of 10⁻⁵ M and 10⁻⁶ M, respectively (either agent alone resulted sometimes in unsustained constriction). Effluent from unstimulated perfused aortic preparations with endothelium was found to dilate coronaries by 17 ± 3% (mean ± s.e., n = 12); when ACh was introduced into the circuit more proximally so as to perfuse also the aorta and

Table 1 Properties of EDRF inhibitors in aortic strips

Agent	IC ₅₀	Interaction with carbonyl groups ⁸⁻¹¹	*Antioxidant ¹²⁻¹⁵	†Nonspecific radical scavengers ^{1,14-16}	‡Phospholipase A ₂ inhibition ^{24,25}	§Lipoxygenase inhibition ^{14,29,30}
Phenylhydrazine	5 × 10 ⁻⁵ M	+				
KBH ₄	3 × 10 ⁻⁴ M	+	+			
Dithiothreitol	2 × 10 ⁻⁴ M	+	+	+		
Cysteine	8 × 10 ⁻⁵ M	+	+	+		
Hydroquinone	4 × 10 ⁻⁶ M		+	+		
Phenidone	10 ⁻⁵ M		+	+		+
Nordihydroguaiaretic acid	10 ⁻⁵ M		+	+		+
Quinacrine	9 × 10 ⁻⁶ M				+	

Agents shown to be EDRF inhibitors were tested against ACh-induced relaxation as described in Fig. 1 legend ($n > 8$ in each case). At the concentrations used these agents had no effect on vasomotor tone in denuded preparations, except slight constriction with $> 7 \times 10^{-4}$ M KBH₄ and relaxation with $> 8 \times 10^{-4}$ M phenylhydrazine, $> 5 \times 10^{-3}$ M dithiothreitol or cysteine, $> 8 \times 10^{-4}$ M hydroquinone, $> 5 \times 10^{-4}$ M phenidone, $> 2 \times 10^{-4}$ M nordihydroguaiaretic acid and $> 10^{-4}$ M quinacrine, which therefore does not affect interpretation of the results.

* KBH₄ specific to carbonyl groups in these conditions¹⁰. This property is theoretically possessed also by quercetin 3 β -D-rutinoside (10^{-4} M), 2',3',4',5,7-pentahydroxyflavone (10^{-4} M), 1,5-dihydroxynaphthalene (10^{-4} M) and oxygen-centred radical scavengers—which did not inhibit EDRF.

† Further nonspecific radical scavengers are the spin trap reagents phenyl-tert-butyl nitron (10^{-4} M)¹⁷ and 2-nitroso-2-methylpropane (10^{-4} M)¹⁷—these did not inhibit EDRF. More specific O₂, OH⁻, O₂⁻ and H₂O₂ scavengers are: 1,4-diazobicyclo(2,2,2)-octane¹⁸ (10^{-4} M), phenylalanine¹⁹ (10^{-3} M), histidine^{19,20} (10^{-3} M), methionine^{19,20} (10^{-3} M), dimethyl sulphoxide²¹ (10^{-4} M), sodium benzoate²² (10^{-2} M), mannitol²² (10^{-2} M), superoxide dismutase²³ (100 U ml⁻¹), catalase²³ (400 U ml⁻¹); these also did not inhibit EDRF.

‡ Possessed also by dexamethasone^{26,27} (10^{-3} M) and *p*-bromophenacyl bromide^{24,25,28} (10^{-5} M) which did not inhibit EDRF.

§ Possessed also by quercetin 3 β -D-rutinoside³¹ (10^{-4} M), 2',3',4',5,7-pentahydroxyflavone³¹ (10^{-4} M) and 1,5-dihydroxynaphthalene³¹ (10^{-4} M), which did not inhibit EDRF.

|| Quinacrine was ineffective against A23187-stimulated EDRF, whereas the other seven effective agents inhibited EDRF stimulated by ACh, ATP, ADP or A23187 ($n > 6$ in each case).

stimulate aortic EDRF, the effluent had a greater dilator effect ($62 \pm 5\%$, $n = 22$, $P < 0.001$; Student's *t*-test) (Fig. 2a, left-hand trace).

The half life of EDRF was calculated as 6.3 ± 0.6 s (Fig. 2) from cascade experiments in which the transit time between aortic and coronary preparations was altered by changing the length of the intervening tubing.

Both basal and stimulated EDRF release were seen only when aortic endothelium was intact, they could be maintained for up to 60 min, were unaffected by preincubation and continuing perfusion of aortae with flurbiprofen (10^{-5} M), and could be inhibited by adding KBH₄, dithiothreitol, phenylhydrazine,

phenidone or nordihydroguaiaretic acid to the proximal end of the intervening tubing (Fig. 3). Infusion of these five agents distally was much less effective: the concentration for half maximal inhibition (IC₅₀) of stimulated EDRF measured from dose responses to the first four agents ($n = 8$ in each case) was 15–50 times greater than with proximal infusion. No such measurement was made for nordihydroguaiaretic acid because it caused an endothelium-independent slow decline in pressure, though its inhibition of EDRF-mediated dilatation was evident before this occurred. By contrast, directly acting constrictor or dilator agents (histamine, 5-HT and glyceryl trinitrate) gave identical dose-responses whether infused proximally or distally.

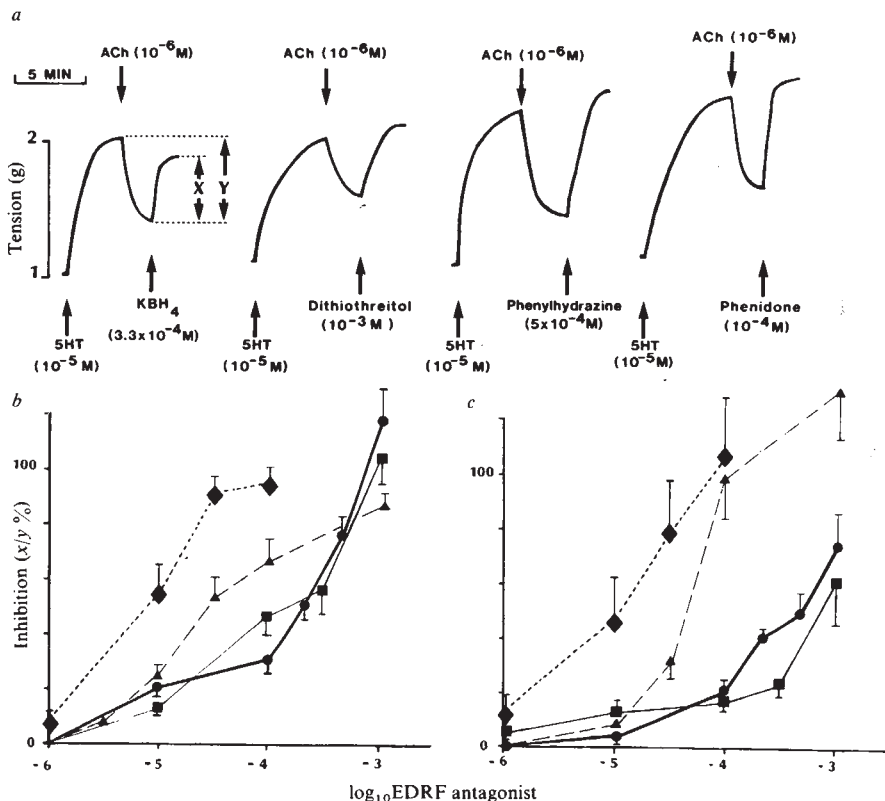


Fig. 1 a, Examples of endothelium-dependent relaxation in aortic strips (constricted by 10^{-5} M 5-HT), using 10^{-6} M ACh to stimulate EDRF and 3.3×10^{-4} M KBH₄, 10^{-3} M dithiothreitol, 5×10^{-4} M phenylhydrazine or 10^{-4} M phenidone to inhibit it. b, c, Dose-response curves for inhibition of EDRF, expressed as x/y (%) ($n > 8$ for each dose response). Preconstriction was induced by 10^{-5} M 5-HT in all cases, and the concentration of EDRF stimulant (10^{-6} M ACh in b or 10^{-7} M A23187 in c) was kept constant while that of the EDRF inhibitors was varied. The four EDRF inhibitors used are KBH₄ (●), dithiothreitol (■), phenylhydrazine (▲) and phenidone (◆). It must be noted that: (1) The EDRF inhibitors illustrated had no direct effect at these concentrations on aortic strips denuded of endothelium, except for $> 7 \times 10^{-4}$ M KBH₄, which sometimes caused slight constriction that may have influenced the final data point in b and c. (2) Maximum EDRF inhibition can be $> 100\%$ due to inhibition of basal EDRF activity⁷ in addition to ACh-stimulated activity. (3) Glucose was omitted from the buffer in experiments with phenylhydrazine with which it combines (lack of this constituent did not affect constrictor responses or EDRF-induced relaxation over the experimental period).

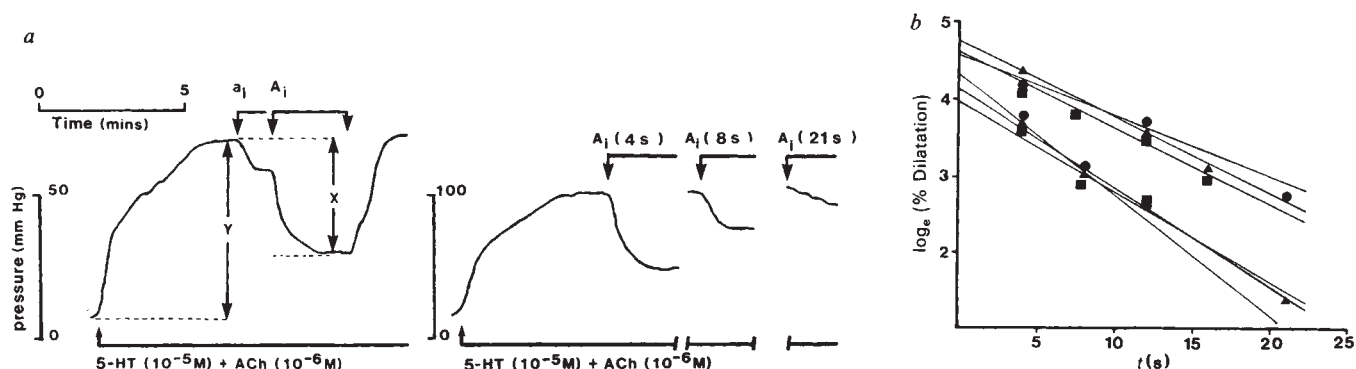


Fig. 2 *a*, Representative pressure recordings from de-endothelialized coronary artery constricted with a combination of 10^{-5} M 5-HT and 10^{-6} M ACh then perfused in cascade from an aorta with intact endothelium. Left-hand trace: Introduction of perfusate from unstimulated aorta (a_1) and from aorta stimulated with 10^{-6} M ACh (A_1); note the increase in pressure on removal of the aorta from the circuit. Dilatation is normalized as $x/y \times 100$. Right-hand traces: Dilatation was inversely related to transit time (t), set by the length of the intervening tubing. *b*, $\log_e$ (% dilatation) plotted against transit time, with linear regression lines for each experiment. In each experiment measurements were made in random order and included more than two determinations at each transit time. Measurements were shown to be similar ($\pm 10\%$) at each transit time whether glass or siliconized polythene was used for the intervening tubing. Mean EDRF half life was 6.3 ± 0.6 s ($n = 6$).

Proximal infusion allowed 4 s interaction with EDRF whereas distal infusion allowed 0.5 s interaction, the coronary preparation being exposed to the same concentration of EDRF inhibitor in each case. These EDRF inhibitors seem, therefore, to be acting by chemical interaction with EDRF in transit, and not on its production or site of action. Their properties thus offer insight into the chemical nature of EDRF.

Table 1 presents the chemical reactions⁸⁻³¹ characteristic of each of the agents tested for EDRF inhibition in aortic strips. EDRF inhibition was demonstrable equally against ACh, ATP or A23187-induced relaxation for all the effective agents listed, except for quinacrine, which we confirmed as being ineffective against A23187 (refs 1, 32). Reversible EDRF inhibition with the first seven of these agents was confirmed also in cascade experiments, in which quinacrine again was ineffective, suggesting that quinacrine acts directly on endothelium. Inactivation of EDRF by phenylhydrazine and KBH_4 indicates that EDRF contains a carbonyl group at or near its active site; the general antioxidant properties common to the other five effective inhibitory agents are consistent with this conclusion. Lipoxigenase

inhibitors were not effective unless they also possessed antioxidant properties. Spin trap reagents and specific oxygen-centred radical scavengers were also ineffective, and a half life of 6 s would, moreover, be long for a biological free radical.

At the concentrations used here, the classes of compounds which inactivate EDRF within 4 s in neutral aqueous buffer at 37°C suggest that EDRF is probably an aldehyde, ketone or lactone. Some lactones which react in such a way are known also to be unstable³³. Other carbonyl-containing compounds would be expected to react slowly or not at all in these circumstances.

We thank Dr P. Shannon for advice on chemical aspects of this study. The British Heart Foundation provided financial assistance. T.M.G. was a British Heart Foundation Junior Research Fellow.

Received 9 December 1983; accepted 6 February 1984.

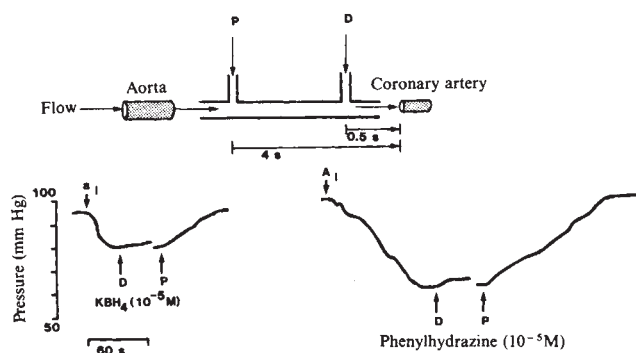


Fig. 3 Agents were infused into the proximal (P) or distal (D) end of the intervening perfusion tubing, representing precoronary interaction times of 4 and 0.5 s respectively. The representative traces show the fall in perfusion pressure of a de-endothelialized coronary artery (precontracted with 10^{-5} M 5-HT and 10^{-6} M ACh) on introducing an endothelialized aorta into the circuit, either unstimulated (a_1 , left trace) or ACh-stimulated (A_1 , right trace). EDRF inhibitors such as KBH_4 (final concentration 10^{-5} M) or phenylhydrazine (final concentration 10^{-5} M) completely inhibited the relaxant effect of effluent from both unstimulated (left-hand traces) and stimulated aortae (right-hand traces) if added proximally but not if added distally.

1. Furchgott, R. F. & Zawadzki, J. V. *Nature* **288**, 373-376 (1980).
2. Furchgott, R. F. *Circulation Res.* **53**, 557-573 (1983).
3. Cherry, P. D., Furchgott, R. F., Zawadzki, J. V. & Jothianandan, D. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2106-2110 (1982).
4. De Mey, J. G., Claeys, M. & Vanhoutte, P. M. *J. Pharmac. exp. Ther.* **222**, 166-173 (1982).
5. Griffith, T. M., Hughes Edwards, D., Lewis, M. J. & Henderson, A. H. *J. molec. cell. Cardiol.* (in the press).
6. Cocks, T. M. & Angus, J. A. *Nature* **305**, 627-630 (1983).
7. Griffith, T. M., Henderson, A. H., Hughes Edwards, D. & Lewis, M. J. *J. Physiol., Lond.* (in the press).
8. Hanna, J. G. *The Chemistry of the Carbonyl Group*, 390-392 (Interscience, London, 1966).
9. Gerrard, W. *The Organic Chemistry of Boron*, 132-160 (Academic, London, 1961).
10. Hajos, A. *Complex Hydrides*, 48-58 (Elsevier, Amsterdam, 1979).
11. Peach, M. E. *The Chemistry of the Thiol Group*, 765-771 (Wiley, London, 1974).
12. Jocelyn, P. C. *Biochemistry of the SH Group*, 55-58 (Academic, New York, 1972).
13. Geissman, T. A. *Principles of Organic Chemistry*, 667-670 (W. H. Freeman, San Francisco, 1968).
14. Robak, J. & Duniec, Z. *Biochem. Pharmac.* **31**, 1955-1959 (1982).
15. Freeman, B. A. & Crapo, J. D. *Lab. Invest.* **47**, 412-426 (1982).
16. Jocelyn, P. C. *Biochemistry of the SH Group*, 330-331 (Academic, New York, 1972).
17. Janzen, E. G. *Free Radicals in Biology* Vol. 4, 115-150 (Academic, London, 1980).
18. Packer, J. E. *et al. Biochem. biophys. Res. Commun.* **98**, 901-906 (1981).
19. Bielski, B. J. H. & Shiue, G. G. *Ciba Fdn Symp.* **65**, 43-56 (1979).
20. Foote, C. S. *Free Radicals in Biology* Vol. 2, 85-133 (Academic, London, 1976).
21. Rosenblum, W. I. *Am. J. Physiol.* **245**, H139-142 (1983).
22. Klebanoff, S. J. & Rosen, H. *Ciba Fdn Symp.* **65**, 263-284 (1979).
23. Fridovich, I. *Science* **201**, 875-880 (1978).
24. Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. & Kato, R. *Biochem. biophys. Res. Commun.* **105**, 759-765 (1982).
25. Yamamoto, S., Nakadate, T., Nakaki, T., Ishii, K. & Kato, R. *Eur. J. Pharmac.* **78**, 225-227 (1982).
26. Flower, R. J. & Blackwell, G. J. *Nature* **278**, 456-459 (1979).
27. Blackwell, G. J. *et al. Nature* **287**, 147-149 (1980).
28. Vallee, E., Gougat, J., Navarro, J. & Delahayes, J. F. *J. Pharm. Pharmac.* **31**, 588-592 (1979).
29. Blackwell, G. J. & Flower, R. J. *Prostaglandins* **16**, 417-425 (1978).
30. Hamberg, M. *Biochim. biophys. Acta* **431**, 651-654 (1976).
31. Baumann, J., Bruchhausen, F. V. & Wurm, G. *Prostaglandins* **20**, 627-639 (1980).
32. Singer, H. A. & Peach, M. J. *J. Pharmac. exp. Ther.* **226**, 796-801 (1983).
33. Stanek, J., Cerny, M., Kocourek, J. & Pacak, J. *The Monosaccharides*, 660-662 (Academic, New York, 1963).

Inhibition of von Willebrand factor-platelet interaction by fibrinogen

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The prolonged bleeding time and frequent haemorrhagic episodes in patients with severe von Willebrand's disease (vWD) attest to the importance of von Willebrand factor (vWF) in platelet adhesive functions. In *in vitro* systems, vWF is directly involved in platelet adhesion at high shear rates, and this participation may be mediated by platelet receptors for vWF^{1,2}. Two apparently distinct mechanisms have now been identified for enhancing ¹²⁵I-vWF interaction with stimulated platelets: one is induced by ristocetin³⁻⁵ and apparently mediated by platelet membrane glycoprotein Ib (GPIb)⁶⁻⁸; a second mechanism has been identified more recently and is induced by the physiological stimuli ADP⁹ and thrombin¹⁰⁻¹¹. The failure of thrombin to support ¹²⁵I-vWF binding to thrombasthenic platelets¹¹, which contain GPIb but lack GPIIb/IIIa, suggests that the mechanism of this interaction may be distinct from ristocetin-induced binding. As ADP and thrombin are released at sites of platelet accumulation, it is possible that these agonists regulate the vWF-platelet interactions *in vivo*. To test this hypothesis, we have examined ADP- and thrombin-supported ¹²⁵I-vWF binding to platelets in the plasma of severe vWD patients, and report here that it is inhibited by fibrinogen. Thus, in addition to its role in coagulation and platelet aggregation, fibrinogen influences the binding of vWF to thrombin- and ADP-stimulated platelets.

Binding of ¹²⁵I-vWF to thrombin- or ADP-stimulated washed platelets was time-dependent and reached a steady state within 30 min at 22 °C (Fig. 1). Consistent with other reports, binding was dependent on stimulus concentration and was maximum at 5 μ M ADP or 0.05 U ml⁻¹ thrombin. Binding of vWF to unstimulated platelets was neither displaced by a large excess of unlabelled vWF nor inhibited by prostaglandin E₁ (PGE₁ at 1 μ M, creatine phosphate/creatine phosphokinase (CP/CPK)

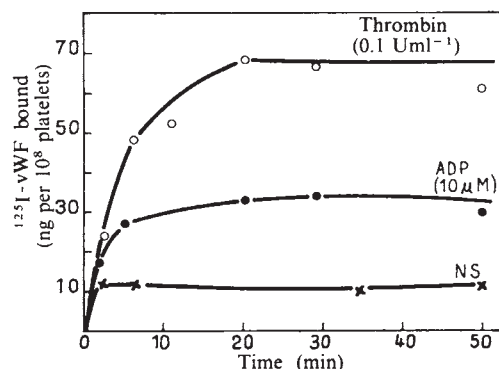


Fig. 1 Time course of ¹²⁵I-vWF binding to thrombin (○) or ADP (●)-stimulated platelets. ×, Binding to non-stimulated (NS) platelets. vWF purified from fresh human plasma¹⁵ was radio-labelled with ¹²⁵I by the IodoGen method¹⁶. Contaminating fibrinogen did not exceed 0.01% as determined by radioimmunoassay. Platelets at 1×10^8 ml⁻¹ in Tyrode-2% albumin buffer¹⁷ were stimulated with ADP or thrombin without stirring at 22 °C in the presence of ¹²⁵I-vWF (5 μ g ml⁻¹). At selected times, 50- μ l aliquots of the platelet suspension were layered onto 350 μ l of 25% sucrose and centrifuged for 3 min at 11,750 r.p.m. in a Beckman microfuge to separate platelet-bound ligand from unbound material. Radioactivity in the tip of the tubes was counted. When thrombin was used as the stimulus, the enzyme was inhibited 2 min after platelet stimulation and immediately before addition of ¹²⁵I-vWF by a 500-fold excess of hirudin. The results represent five different experiments on platelets from separate donors.

at 25.5 and 0.5 mg ml⁻¹, respectively, or EDTA at 1 mM. It was thus nonspecific and subtracted in subsequent experiments. Specific binding was divalent ion-dependent, displaced by excess unlabelled vWF and inhibited by PGE₁ and CP/CPK as reported previously¹⁰. Autoradiography of platelet-associated ¹²⁵I on SDS-agarose gels¹² revealed that the platelet-bound vWF had the same multimeric structure as the added ¹²⁵I-vWF.

To study the interaction of ¹²⁵I-vWF with platelets in physiological conditions, binding was measured in the platelet-rich plasma from two patients with severe vWD. With both ADP and thrombin, ¹²⁵I-vWF interacted normally with vWD washed platelets (Fig. 2). In contrast, binding in plasma was reduced by 80% with ADP and by 82% with thrombin (Fig. 2). Identical

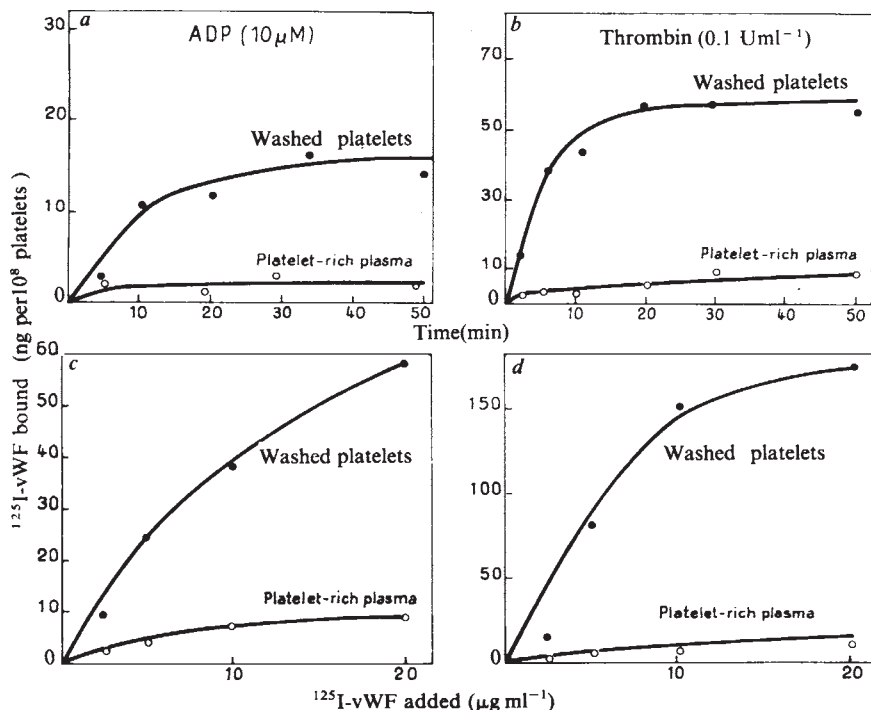


Fig. 2 ¹²⁵I-vWF binding to ADP (a, c) or thrombin (b, d) stimulated vWD platelets in buffer or in plasma. Blood was collected from a patient with severe vWD and ¹²⁵I-vWF binding to washed platelets (●) or platelet-rich plasma (PRP, ○) of the patient was measured as a function of time (a, b) or vWF concentration (c, d). This patient had $<4 \times 10^{-5}$ U ml⁻¹ of VWF antigen in plasma and platelets and normal plasma and platelet fibrinogen levels. In both cases the platelet concentration was 1×10^8 ml⁻¹, and the citrated platelet-poor plasma (PPP) of the same patient was used to adjust the PRP concentration to this level. ¹²⁵I-vWF was present at 5 μ g ml⁻¹. In the experiment shown with thrombin as the stimulus, Gly-Pro-Arg-Pro was included at 1 mM concentration to prevent clot formation in the plasma¹⁸; the tetrapeptide did not affect ¹²⁵I-vWF binding to ADP- or thrombin-stimulated washed platelets.

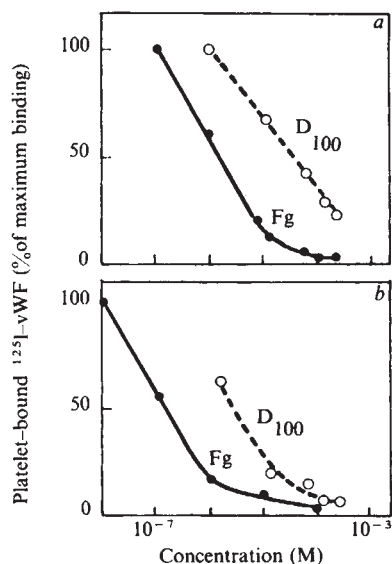


Fig. 3 Inhibition by fibrinogen (Fg) or fragment D100 of ^{125}I -vWF binding to thrombin(a) or ADP(b)-stimulated platelets. Washed platelets ($1 \times 10^8 \text{ ml}^{-1}$) were stimulated with 0.1 U ml^{-1} thrombin or $10 \mu\text{M}$ ADP in the presence of $5 \mu\text{g ml}^{-1}$ ^{125}I -vWF and varying concentrations of competitor. Binding was determined after incubation for 50 min at 22°C . The results are calculated as per cent of ^{125}I -vWF bound to the platelets in the absence of competitor.

results were observed for both patients. The failure of ^{125}I -vWF to bind in plasma was independent of the anticoagulant used since similar results were obtained in heparinized plasma (not shown). In addition, this decreased binding was not reversed by addition of 0.5 mM CaCl_2 to citrated plasma and concentrations of citrate similar to those of anticoagulated plasma did not inhibit the binding of vWF to washed platelets in buffer systems, thus excluding an indirect effect of citrate on binding. Corroborating this conclusion is the observation that ^{125}I -vWF binding to platelets in citrated plasma from afibrinogenemic patients is normal¹³.

Based on the ability of thrombin and ADP to support both vWF and fibrinogen binding to platelets, we considered the possibility that inhibition of vWF binding in plasma was due to fibrinogen. Indeed, binding of ^{125}I -vWF to ADP- or thrombin-stimulated platelets was markedly inhibited by 10^{-5} M fibrinogen and plasmin fragment D100 (molecular weight (MW) 100,000) (Table 1). In contrast, fragments D80 (MW 80,000) and E50 (MW 50,000) had no effect. Inhibition by fibrinogen or fragment D100 was not observed with ristocetin-induced vWF binding and was dose-dependent for ADP and thrombin (Fig. 3). At physiological concentrations of fibrinogen ($0.5\text{--}1 \times 10^{-5} \text{ M}$), ^{125}I -vWF binding was inhibited $>80\%$ with both stimuli. These results are in contrast to those of Fujimoto *et al.*¹⁰ and demonstrate that fibrinogen prevents the interaction of vWF with thrombin- and ADP-stimulated platelets in buffer and in plasma. It is suggested that vWF binding to platelets is not involved in platelet aggregation in physiological conditions.

Table 1 Effect of purified¹⁴ fibrinogen and its plasmin derivatives (10^{-5} M) on binding of ^{125}I -vWF to stimulated platelets

Addition to platelets	Inhibition of ^{125}I -vWF binding (%)		
	ADP ($10 \mu\text{M}$)	Thrombin (0.1 U ml^{-1})	Ristocetin (1 mg ml^{-1})
Fibrinogen	90	87	0
D100	70	34	0
D80	0	ND	ND
E50	11	0	0

ND, not determined.

We thank E. F. Plow and M. Ginsberg (Research Institute of Scripps Clinic) for stimulating discussions and suggestions during the preparation of this manuscript.

Received 20 July 1983; accepted 30 January 1984.

- Weiss, H. J., Turritto, V. T. & Baumgartner, H. R. *J. Lab. clin. Med.* **92**, 750–764 (1974).
- Meyer, D. & Baumgartner, H. R. *Br. J. Haemat.* **54**, 1–9 (1983).
- Zucker, M. B., Kim, J. J., McPherson, J. & Grant, R. A. *Br. J. Haemat.* **35**, 535–549 (1977).
- Kao, K. J., Pizzo, S. V. & McKee, P. A. *J. clin. Invest.* **63**, 656–664 (1979).
- Morisato, D. K. & Gralnick, H. R. *Blood* **55**, 9–15 (1980).
- Moake, J. L. *et al. Thromb. Res.* **19**, 21–27 (1980).
- Jenkins, C. S. P. *et al. J. clin. Invest.* **57**, 112–124 (1976).
- Ruan, C. *et al. Br. J. Haemat.* **49**, 511–519 (1981).
- Fujimoto, T. & Hawiger, J. *Nature* **297**, 154–156 (1982).
- Fujimoto, T., Ohara, S. & Hawiger, J. *J. clin. Invest.* **69**, 1212–1222 (1982).
- Ruggeri, Z. M., Bader, R. & de Marco, L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 6038–6041 (1982).
- Ruggeri, Z. M. & Zimmerman, T. S. *Blood* **57**, 1140–1143 (1981).
- Montgomery, R. R., Schulleck, J. & Jordan, J. *Blood* **62** (Suppl. 1), 263a (1983).
- Marguerie, G. A., Ardailou, N., Cherel, G. & Plow, E. F. *J. biol. Chem.* **257**, 11872–11875 (1982).
- Meyer, D., Obert, B., Pietu, G., Laverne, J. M. & Zimmerman, T. S. *J. Lab. clin. Med.* **95**, 590–602 (1980).
- Fraker, P. & Speck, J. C. Jr *Biochem. biophys. Res. Commun.* **80**, 849–857 (1978).
- Marguerie, G. A., Plow, E. F. & Edgington, T. S. *J. biol. Chem.* **254**, 5357–5363 (1979).
- Laudano, A. P. & Doolittle, R. F. *Proc. natn. Acad. Sci. U.S.A.* **75**, 3085–3089 (1978).

Is renin substrate an erythropoietin precursor?

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The biogenesis of erythropoietin is incompletely understood^{1,2}. One hypothesis maintains that erythropoietin is synthesized primarily in the kidney³ while according to another theory an erythropoietin precursor present in plasma is activated by a renal factor, erythrogastrin⁴. An attractive candidate for the erythropoietin precursor is renin substrate (angiotensinogen) which has chemical similarities with erythropoietin⁵. We show here that purified renin substrate from human plasma is immunologically related to human erythropoietin. Moreover, purified renin substrate, like erythropoietin, causes the dose-dependent increase of haemoglobin F in cultured human erythroid leukaemia K562 cells. We conclude that renin substrate is a likely precursor of erythropoietin.

Erythropoietin (EP)⁶ and renin substrate (RS)⁷ are both acidic glycoproteins, with an isoelectric point close to 4.5 and have similar sugar moieties and amino acid compositions. The molecular weight (MW) of EP is $\sim 33,000$ (ref. 8) and that of RS $\sim 56,800$ (ref. 7). Several recent reports⁹ provide evidence that, at least under certain circumstances, human liver may produce EP or a precursor of EP. For example, acute viral hepatitis¹⁰ or hepatotoxic drugs may cause erythrocytosis in chronic renal failure and even in anephric patients.

To test the hypothesis that RS is related to EP we compared the immunological and biological properties of these substances. RS purified to homogeneity from pooled human plasma was used as an antigen in a quantitative haemagglutination inhibition test for human EP¹¹. The RS preparation used yielded one band on SDS gel electrophoresis and contained $12.0 \mu\text{g}$ angiotensin I per mg protein, as compared with $10.8 \mu\text{g}$ per mg protein for a highly purified RS preparation⁷ and the theoretical maximum of $22.8 \mu\text{g}$ per mg protein based on a MW of 56,800 for RS⁷. The reaction of renin substrate was comparable to purified human EP (Table 1), corresponding to 9,600 milli-immunochemical units per mg protein. Various control proteins showed no such immunoreactivity. Two antisera against human plasma RS raised in rabbits caused agglutination of human erythrocytes covered with glutaraldehyde-coupled human EP (Table 2) comparable to the agglutination caused by rabbit anti-human EP. In addition, antiserum against human RS could replace anti-

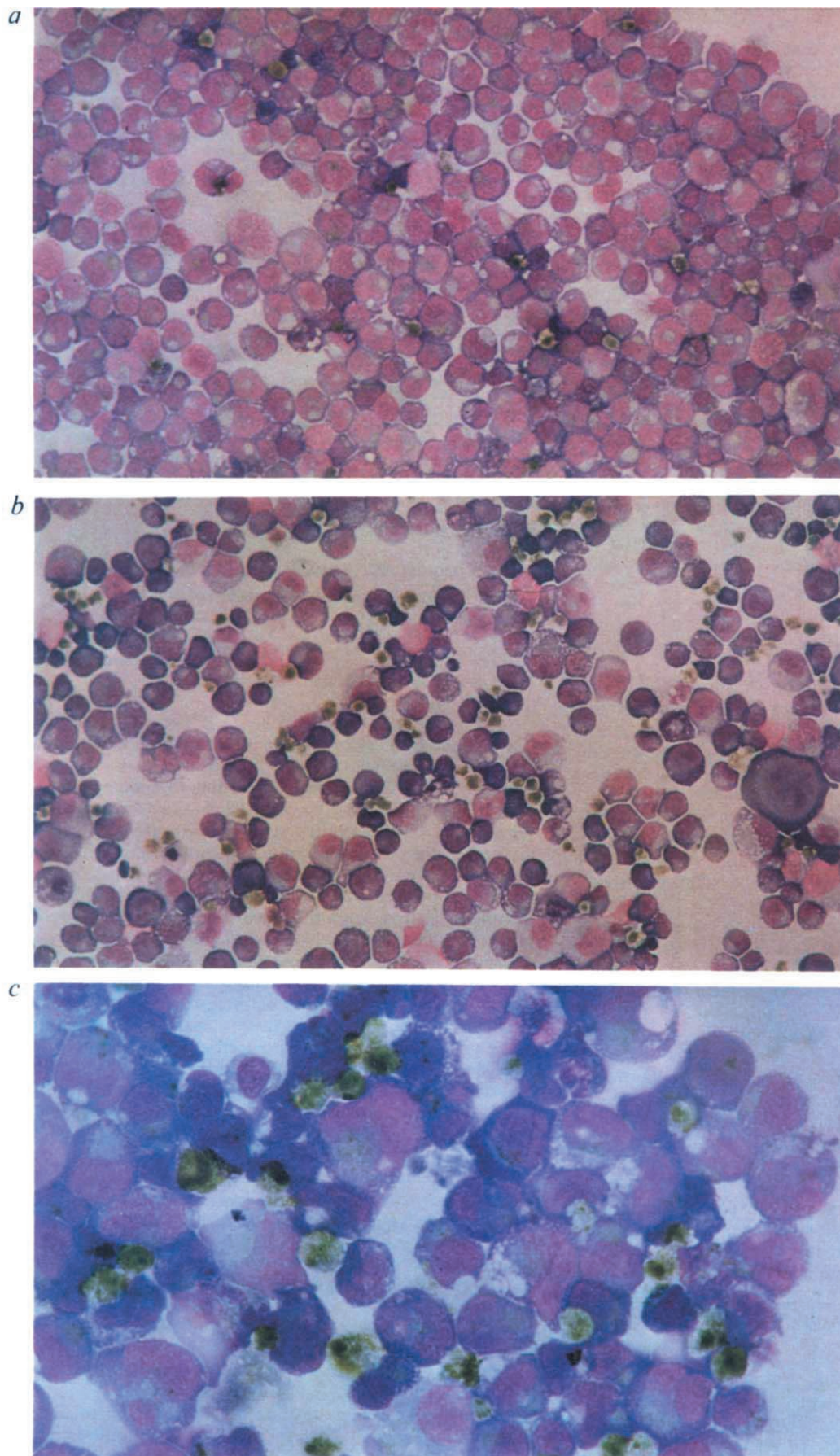


Fig. 1 *a*, Photomicrographs of K562 cells cultured for 10 days in the presence of $20\ \mu\text{M}$ haemin and stained with May-Grünwald-Giemsa stain and by the Lephnes benzidine method. *b*, Addition of $200\ \text{mU ml}^{-1}$ erythropoietin (Connaught III, Connaught Laboratories) and $20\ \mu\text{M}$ haemin caused a marked increase in benzidine-positive buds. *c*, A similar effect was observed using $30\ \mu\text{g ml}^{-1}$ renin substrate and $20\ \mu\text{M}$ haemin. K562 cells were cultured in RPMI 1640 culture medium containing 10% fetal calf serum (details to be published elsewhere).

Table 1 Immunoreactivity of human plasma renin substrate in haemagglutination inhibition assay for human erythropoietin

Substance tested	No. of expts	Erythropoietin	
		Immunoactivity measured (mU per mg protein; mean)	Bioactivity stated
Renin substrate	5	9,600	
Erythropoietin, 2nd International Reference preparation*	4	1,200	2,000
Sheep erythropoietin†	4	0	1,400
Human kidney renin	3	0	
Angiotensin I	3	0	
Angiotensin II	3	0	

In addition, the following substances did not cross-react in the erythropoietin haemagglutination inhibition assay¹¹ (JCL Clinical Research Corporation, Knoxville, Tennessee): anti-granulocyte colony stimulating activity, human γ -globulin, human transferrin, ovalbumin, chymotrypsinogen A, human α_1 -antitrypsin, cytochrome c, human haemoglobin, myoglobin, coeluroplasmin and α_2 -macroglobulin. Renin substrate (RS) was purified from pooled Na₂EDTA-plasma of pregnant women via the following steps: ammonium sulphate precipitation, cellulose DEAE-52 chromatography, Blue Sepharose CL-6B (Pharmacia) chromatography, Ultrogel AcA 34 filtration (LKB), chromatofocusing (Pharmacia) and Sephadex G-75 gel filtration. The purified RS preparation yielded one band on SDS-gel electrophoresis, and 12.0 μ g angiotensin I per mg protein on exhaustive incubation with human kidney renin (details to be published elsewhere). Renin was partially purified from human kidney according to Haas *et al.*¹⁶.

* Human urinary erythropoietin, 2.0 international units per mg protein (WHO International Laboratory for Biological Standards; National Institute for Biological Standards and Control, London).

† Connaught Step 3, Connaught Laboratories).

human EP antiserum in the haemagglutination inhibition assay for human EP. Finally, antisera to human EP were shown to bind ¹²⁵I-labelled RS in a dose-dependent manner. For example, one anti-human EP antiserum (Fisher) bound 29.3, 11.2 and 3.6% of a constant amount of ¹²⁵I-RS when diluted 1:30, 1:100 or 1:1,000 respectively. The maximum binding of anti-RS antiserum to ¹²⁵I-RS was 78.3% at a dilution of 1:100. Antisera against angiotensin I, purified human lung angiotensin converting enzyme or haemoglobin F showed no binding of labeled RS. These results indicate considerable immunological similarity between human plasma renin substrate and erythropoietin.

To test whether RS can exert erythropoietin-like activity, cultured human leukaemia cells of the K562 line were treated with RS. This cell line is known to react to erythropoietin treatment with increased numbers of benzidine-positive colonies¹² and displays properties characteristic of early erythroid progenitor cells¹³. Treatment with RS for 10 days caused a dose-dependent increase in benzidine-positive buds in cultured K562 cells (Fig. 1) that could not be distinguished from the effect of purified sheep EP.

The effect of RS on haemoglobin synthesis in K562 cells was further evaluated using a radioimmunoassay for haemoglobin F (HbF). RS caused a dose-dependent increase of immunoreactive HbF in K562 cells (Fig. 2), while purified human kidney renin or angiotensin I and II had no such effect. The stimulation of HbF production by K562 cells caused by RS treatment was comparable to the effect of EP, and strongly suggests that RS has erythropoietin-like bioactivity, either by a direct action on K562 cells or following chemical modification by these cells or factors present in the culture medium.

Taken together, the present data provide evidence that renin substrate is closely related to erythropoietin and is a likely precursor of this hormone. We are currently investigating whether renal factors like erythropoietin⁴ participate in the biogenesis of EP by an action on RS, and whether normal erythroid progenitor cells are activated by RS. The immunological

Table 2 Agglutination of erythrocytes covered with human erythropoietin by antisera against human renin substrate and erythropoietin

Antiserum	No. of expts	Maximal dilution showing agglutination
Rabbit anti-human renin substrate		
-6/032083*	4	1:640
-13/032083	4	1:1,280
Rabbit anti-human erythropoietin†	3	1:800
Rabbit control sera		
Non-immunized	4	Negative
Anti-human HbA	3	Negative
Anti-human HbF	3	Negative

Agglutination is defined as in the haemagglutination inhibition assay for human erythropoietin (JCL Clinical Research Corporation, Knoxville). Antisera against human RS were generated by monthly subcutaneous and intradermal injections of purified RS (Table 1) with complete Freund's adjuvant in rabbits.

* Code number/date of bleeding.

† Antiserum against purified human erythropoietin was a gift of Dr J. W. Fisher and has been described elsewhere¹⁷. This antiserum was capable of neutralizing the biological activity of human erythropoietin in the exhypoxic polycythaemic mouse bioassay. It was prepared using the pure preparation of EP from human urine of Miyake *et al.*¹⁸, containing 70,000 U per mg protein.

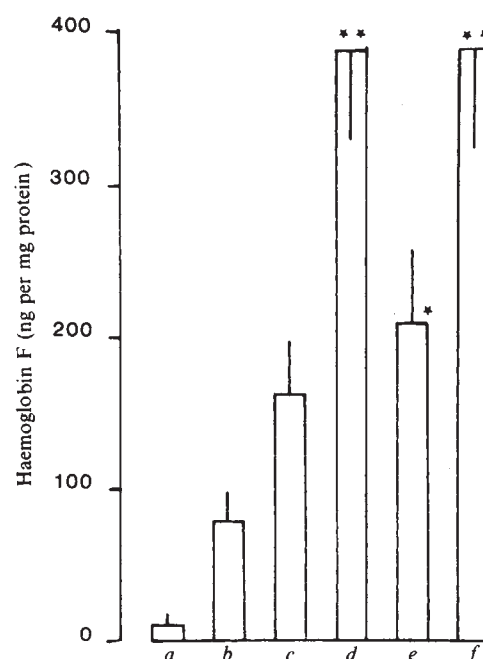


Fig. 2 Haemoglobin F (HbF) concentration in lysed K562 cells: a, without treatment; b, after treatment for 10 days in culture with 20 μ M haemin (haemin control); plus c, 20 μ g ml⁻¹ renin substrate; d, 50 μ g ml⁻¹ renin substrate; e, 20 mU ml⁻¹ sheep erythropoietin; and f, 200 mU ml⁻¹ sheep erythropoietin. Cells were cultured in RPMI 1640 medium containing 10% fetal calf serum, 200 IU ml⁻¹ penicillin and 200 μ g ml⁻¹ streptomycin at 37 °C and 5% CO₂. Pretreatment with 20 μ M haemin for 3 days was followed by treatment for 10 days with the substances mentioned. Cells were washed and lysed by repeated freezing to -70 °C and thawing in 100 mM KCN. HbF was quantitated by radioimmunoassay (K.R. *et al.*, in preparation) using ¹²⁵I-HbF purified from cord blood according to the International Committee for Standardization of Haematology as a standard. Following carbodiimide condensation purified HbF was used as an immunogen to raise antisera in rabbits. The assay sensitivity was <0.2 ng HbF and the cross-reaction with human haemoglobin A 0.8% (not detectable with rabbit haemoglobin). Results are mean $\pm$ s.e.m. Difference from haemin control: * $P < 0.05$; ** $P < 0.01$ (3–5 cell batches; 4–6 assays).

between serum EP assay results obtained with bioassay and immunoassay¹⁴, the latter approach usually giving higher EP values than the former¹⁵. Our data challenge the generally held view that the sole role of renin substrate is to release angiotensin I when acted on by renin.

This work was supported by the MRC of Finland, by the Sigrid Jusélius Foundation, Helsinki, and by the Nordisk Insulin-fond, Copenhagen.

Received 3 July 1983; accepted 10 January 1984.

- Spivak, J. L. & Graber, S. E. *Johns Hopkins med. J.* **146**, 311–320 (1980).
- Fisher, J. W. & Busuttil, R. W. in *Kidney Hormones* Vol. 2 (ed. Fisher, J. W.) 165–185 (Academic, New York, 1977).
- Jacobson, L. O. *et al. Trans. Ass. Am. Physicians* **70**, 305–317 (1957).
- Gordon, A. S. & Kaplan, S. M. in *Kidney Hormones* Vol. 2 (ed. Fisher, J. W.) 187–229 (Academic, New York, 1977).
- Gould, A. B., Goodman, S., De Wolf, R., Onesti, G. & Swartz, C. J. *Lab. clin. Med.* **96**, 523–534 (1980).
- Espada, J. in *Kidney Hormones* Vol. 2 (ed. Fisher, J. W.) 37–71 (Academic, New York, 1977).
- Tewksbury, D. A. & Dart, R. A. *Molec. cell. Biochem.* **27**, 47–56 (1979).
- Shelton, R. N., Ichiki, A. T. & Lange, R. D. *Biochem. Med.* **12**, 45–54 (1975).
- Meyrier, A., Simon, P., Boffa, G. & Brissot, P. *Nephron* **29**, 3–6 (1981).
- Simon, P., Meyrier, A., Tanquerel, T. & Ang, K-S. *Br. med. J.* **280**, 892–898 (1980).
- Lange, R. D. & Ichiki, A. T. in *Kidney Hormones* Vol. 2 (ed. Fisher, J. W.) 111–149 (Academic, New York, 1977).
- Hoffman, R. *et al. Blood* **54**, 1182–1187 (1979).
- Andersson, L. C., Jokinen, M. & Gahmberg, C. G. *Nature* **278**, 364–365 (1979).
- Sherwood, J. & Goldwasser, E. *Blood* **54**, 885–893 (1979).
- Cotes, P. M. *Br. J. Haemat.* **59**, 427–438 (1982).
- Haas, E. *et al. Circulation Res.* **19**, 739–745 (1966).
- Rege, A. B., Brookins, J. & Fisher, J. W. *J. Lab. clin. Med.* **100**, 829–843 (1982).
- Miyake, T., Kung, C. K-H. & Goldwasser, E. *J. biol. Chem.* **252**, 5558–5564 (1977).

Carboxy terminus of vasopressin required for activity but not binding

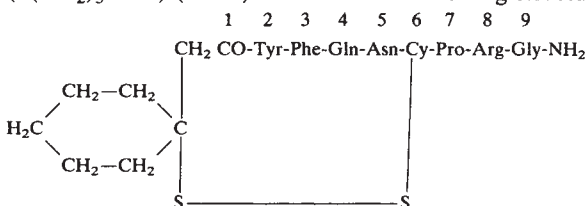
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Vasopressin antagonists^{1–7} are valuable pharmacological tools for investigating physiological and behavioural functions of the nonapeptide arginine-vasopressin (AVP)^{8,9}. The removal of glycineamide from the carboxy terminus of AVP drastically reduces its characteristic vasopressor and antidiuretic activities¹⁰. In contrast to this we show here that removal of the carboxy-terminal glycineamide or the glycine at position 9 from several vasopressin antagonists makes little difference to their ability to block vasopressor and antidiuretic responses to AVP. These data demonstrate the critical structural requirements of the carboxy-terminal position for receptor activation, in contrast to the lack of such requirements for receptor binding. They also provide an avenue to a wide variety of antagonists substituted at the carboxy terminus (for example radiolabelled derivatives and affinity ligands) and suggest clues for the development of more potent and/or selective antagonists.

Removal by tryptic digestion of the carboxy-terminal glycineamide from AVP virtually abolishes its characteristic antidiuretic and vasopressor activities¹⁰. However desglycinamide-AVP does retain activity in behavioural tests in rats¹¹ and a desglycinamide metabolite of AVP has recently been reported to be substantially more potent than AVP in facilitating memory consolidation in rats¹². We wondered how this modification and also the deletion of glycine alone (thereby preserving the CONH₂ portion of the carboxy terminus) would affect the antagonistic potencies of AVP vasopressor and renal tubular receptor antagonists^{1–7}. Initially we synthesized the desglycinamide and desglycine analogues of the vasopressor antagonist/weak antidiuretic agonist [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine vasopressin

(d(CH₂)₅AVP) (ref. 2) which has the following structure:



and the antidiuretic/vasopressor antagonist d(CH₂)₅[D-Phe²]VAVP (ref. 6). This differs from d(CH₂)₅AVP in having D-Phe at position 2 and Val at position 4 in place of Tyr and Gln, respectively. The desglycine analogues of three additional potent antidiuretic/vasopressor antagonists, d(CH₂)₅[Tyr-(Et)²]VAVP (refs 3 and 4) d(CH₂)₅[D-Tyr(Et)]VAVP (ref. 5) and d(CH₂)₅[D-Phe², Ile⁴]AVP (ref. 7), were also synthesized and examined (Table 1). We also report the pharmacological activities of desglycine-AVP for the first time and those of desglycinamide-AVP¹⁰ and of AVP-acid¹³ in more detail than previously published.

Antidiuretic and vasopressor agonistic activities of the AVP analogues were assayed by intravenous injection into ethanol-anaesthetized water-loaded rats¹⁴ and rats pretreated with phenoxybenzamine and under urethane anaesthesia¹⁵, respectively. The same types of assay preparations were used to estimate antagonism of vasopressor¹⁶ and antidiuretic^{3,4} responses to AVP. Antagonistic activities are expressed in terms of the 'effective dose' which is defined as the dose of an antagonist, injected intravenously, that would halve the response to AVP relative to its response before antagonist administration. The effective dose was estimated by finding doses both above and below the effective dose which was then estimated by interpolation on a logarithmic scale¹⁷.

Deletion of glycineamide or glycine from d(CH₂)₅AVP and from d(CH₂)₅[D-Phe²]VAVP resulted in compounds (1a, 1b and 2a, 2b, Table 1) which retained almost full or enhanced antagonistic potencies. In these antagonists, the desglycine modification appears to be more effective in leading to retention or enhancement of antagonistic potencies than the desglycinamide modification. Deletion of glycine from the three additional potent antagonists of both antidiuretic and vasopressor responses (compounds 3–5, Table 1) resulted in compounds (3a–5a) which are almost indistinguishable from their parent glycine-containing compounds. If one assumes that antagonistic potency depends on the ability of a peptide to bind to receptors without activating them, it would appear first that deletion of glycine does not affect the binding of these peptides to renal tubular or to vasopressor receptors and second that deletion of glycineamide reduces binding to renal tubular and vascular receptors only slightly. Note also that deletion of glycine or glycineamide abolished the residual weak transient antidiuretic activity seen in compounds 2, 3 and 4.

As expected, desglycinamide-AVP¹⁰ and AVP-acid¹³ have markedly reduced antidiuretic activities (Table 1). Vasopressor agonistic activity was very weak or undetectable. Neither analogue showed clear antagonistic activity on either assay. Surprisingly, however, desglycine-AVP did have substantial antidiuretic activity, about 50% that of AVP itself. It acted as a weak partial agonist and antagonist of the vasopressor response to AVP. Thus, although removal of the glycine alone in desglycine-AVP did reduce antidiuretic activity by about 50%, and essentially eliminated vasopressor activity, the effects of deleting the carboxamide groups (by deamidation, in AVP-acid, or by deleting the glycineamide, in desglycinamide-AVP) were even more dramatic, reducing antidiuretic activity by about 98%. Thus, these data, in conjunction with those on the AVP antagonists, indicate that neither the carboxy-terminal carboxamide group nor glycineamide is required for binding to either AVP renal tubular or vascular receptors. On the other hand a carboxy-terminal carboxamide group is critical for activation of AVP-renal tubular receptors and the entire glycineamide residue is essential for effective activation of AVP vasopressor receptors.

Table 1 Agonistic and antagonistic activities of some analogues of arginine-vasopressin

Analogue	Agonistic activities (units mg ⁻¹)		Antagonistic activities effective dose (nmol kg ⁻¹)	
	Antidiuretic	Vasopressor	Antidiuretic	Vasopressor
AVP (ref. 1)	330 ± 23	382 ± 5	—	—
AVP-acid*	4.7 ± 0.6	< 0.3	—	—
desGly ⁹ AVP†	164 ± 4	< 0.05§	—	60 ± 16
desGly ⁹ (NH ₂)AVP†	5.6 ± 1.1	~ 0.02§	—	—
1 d(CH ₂) ₅ AVP (ref. 2)	0.03 ± 0.01	—	—	0.56 ± 0.11
1a desGly ⁹ -d(CH ₂) ₅ AVP†	~ 0.003§	—	—	0.28 ± 0.04
1b desGly(NH ₂) ⁹ -d(CH ₂) ₅ AVP†	0.04 ± 0.01	—	—	0.73 ± 0.07
2 d(CH ₂) ₅ [D-Phe ²]VAVP (ref. 6)	Weak§	—	0.67 ± 0.13	0.58 ± 0.04
2a desGly ⁹ -d(CH ₂) ₅ [D-Phe ²]VAVP†	—	—	0.58 ± 0.11	0.47 ± 0.04
2b desGly(NH ₂) ⁹ -d(CH ₂) ₅ [D-Phe ²]VAVP†	—	—	1.30 ± 0.35	0.80 ± 0.08
3 d(CH ₂) ₅ [Tyr(Et) ²]VAVP (refs 3-4)	~ 0.03§	—	1.9 ± 0.2	0.49 ± 0.11
3a desGly-d(CH ₂) ₅ [Tyr(Et) ²]VAVP†	—	—	1.0 ± 0.2	0.45 ± 0.02
4 d(CH ₂) ₅ [D-Tyr(Et) ²]VAVP (ref. 5)	Weak§	—	1.1 ± 0.2	0.45 ± 0.11
4a desGly-d(CH ₂) ₅ [D-Tyr(Et) ²]VAVP†	—	—	1.8 ± 0.3	0.45 ± 0.04
5 ¹ (CH ₂) ₅ [D-Phe ² , Ile ⁴]AVP (ref. 7)	—	—	0.46 ± 0.07	0.99 ± 0.12
5a desGly ⁹ -d(CH ₂) ₅ [D-Phe ² , Ile ⁴]AVP†	—	—	0.66 ± 0.17	1.0 ± 0.1

Except for AVP-acid, which was purchased from Bachem, all AVP analogues were synthesized in our laboratories by standard methods of peptide synthesis either in solution or by the Merrifield solid-phase method¹⁸, as previously described²⁻⁷. Details of these syntheses will be reported elsewhere.

* This analogue was originally reported to be an antidiuretic antagonist *in vitro* and *in vivo*¹³, but is shown here to be an antidiuretic agonist *in vivo*.

† This publication.

‡ Originally obtained by tryptic cleavage of AVP¹⁰, pharmacological properties reported here for the first time.

§ These analogues showed weak partial agonistic activity in these assays which was inconsistent and not clearly related to dose. Abbreviations: AVP, arginine-vasopressin; AVP-acid, deamidoarginine vasopressin or vasopressinoic acid; d(CH₂)₅AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin; d(CH₂)₅[Tyr(Et)²]VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-*o*-ethyltyrosine, 4-valine]arginine-vasopressin; d(CH₂)₅[D-Tyr(Et)²]VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-(*o*-ethyl)tyrosine, 4-valine]arginine-vasopressin; d(CH₂)₅[D-Phe²]VAVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-phenylalanine, 4-valine]arginine-vasopressin; d(CH₂)₅[D-Phe², Ile⁴]AVP, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-phenylalanine, 4-isoleucine]arginine-vasopressin (carboxy terminus is Arg(NH₂); desGly, desglycine; desGly(NH₂), desglycinamide (carboxy terminus is Arg(OH)).

Radiolabelled, photoaffinity or immunogenic covalent ligands of AVP receptor antagonists have not been developed to date. Such ligands could be useful tools for probing the structural characteristics and binding specificities of AVP receptors, isoreceptors and for the production of AVP anti-idiotypic antibodies. The desglycinamide modification of AVP receptor antagonists presents a novel and simple pathway for the synthesis of such ligands; by chemical or enzymatic coupling, in solution or on a solid support of a desglycinamide analogue via its carboxy-terminal COOH group to the amino group of an appropriately radiolabelled amino acid, photoaffinity derivatives or of suitable haptens.

Based on the data presented here for the AVP antagonists and on the finding that the Gly at position 9 in d(CH₂)₅[D-Phe², Ile⁴]AVP (ref. 7) can be replaced by L- or D-Ala, Ser or Arg, with retention of antagonism (unpublished), it is clear that position 9 can be either deleted or substituted with a variety of other derivatives with retention of potent antagonism. This opens up the possibility of incorporating modifications at this position which might lead to more potent and/or more selective antagonists for use as pharmacological tools in studies on the physiological, pathophysiological and behavioural roles of AVP and for potential clinical use.

We thank Ms Beverley Cifelli and Ms Donna Freshour for their help in manuscript preparation. This work was supported in part by research grants from the National Institute of General Medical Sciences (GM25280) and the National Institute of Arthritis, Diabetes, and Digestive and Renal Diseases (AM-01940). The following authors are visiting investigators; A.O. from the Technical University of Lodz, Poland; W.K. and E.N. from the University of Wroclaw, Poland; A.K. from the Technical University of Gdansk, Poland; and A.M. from the University of Warsaw, Poland.

Received 2 November 1983; accepted 11 January 1984.

1. Sawyer, W. H., Grzonka, Z. & Manning, M. *Molec. cell. Endocr.* **22**, 117-134 (1981).
2. Kruszynski, M. *et al. J. med. Chem.* **25**, 364-368 (1980).
3. Sawyer, W. H. *et al. Science* **212**, 49-51 (1981).
4. Manning, M., Lammek, B., Kolodziejczyk, A. M., Seto, J. & Sawyer, W. H. *J. med. Chem.* **24**, 701-706 (1981).

5. Manning, M. *et al. J. med. Chem.* **25**, 45-50 (1982a).
6. Manning, M., Klis, W. A., Olma, A., Seto, J. & Sawyer, W. H. *J. med. Chem.* **25**, 414-419 (1982).
7. Manning, M., Olma, A., Klis, W. A., Seto, J. & Sawyer, W. H. *J. med. Chem.* **26**, 1607-1613 (1983).
8. Reid, I. A. & Schwartz, J. in *Frontiers in Neuroendocrinology* Vol. 8 (eds Martini, L. & Ganong, W. F.) 177-197 (Raven, New York, 1984).
9. Manning, M. & Sawyer, W. H. *Trends Neurosci.* **7**, 6-9 (1984).
10. du Vigneaud, V., Lawler, H. C. & Popenoe, E. A. *J. Am. chem. Soc.* **75**, 4880-4881 (1953).
11. Kovacs, G. L. & de Wied, D. *Neuroendocrinology* **37**, 258-261 (1983).
12. Burbach, J. P. H., Kovacs, G. L., de Wied, D., van Nispen, J. W. & Greven, H. M. *Science* **221**, 1310 (1983).
13. Dousa, T., Hechter, O., Walter, R. & Schwartz, I. L. *Science* **167**, 1134-1135 (1970).
14. Sawyer, W. H. *Endocrinology* **63**, 694-698 (1958).
15. Dekanski, J. *Br. J. Pharmac.* **7**, 567-572 (1952).
16. Dyckes, D. F., Nestor, J. J. Jr, Ferger, M. F. & du Vigneaud, V. *J. med. Chem.* **17**, 250-252 (1974).
17. Schild, H. O. *Br. J. Pharmac. chem. Ther.* **2**, 189-206 (1947).
18. Barany, G. & Merrifield, R. B. in *The Peptides* Vol. 2 (eds Gross, E. & Meienhofer, J.) 1-284 (Academic, New York, 1979).

Release of the predicted calcitonin gene-related peptide from cultured rat trigeminal ganglion cells

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Calcitonin gene-related peptide (CGRP) is a putative novel neuropeptide predicted on the basis of alternative RNA processing events of primary transcripts of the calcitonin gene¹. Distinct mRNAs encoding either calcitonin or CGRP are generated from the calcitonin gene RNA transcript in what appears to be a tissue-specific manner^{1,2}. The predicted peptide has now been detected immunocytochemically in discrete regions of the central and peripheral nervous systems² and potent *in vivo*

actions have been reported for centrally and peripherally administered synthetic CGRP³. However, so far there is no evidence that CGRP is secreted or released by intact cells. The present experiments investigated the possible secretion of CGRP *in vitro* using primary dispersed cell cultures of the adult rat trigeminal ganglion, which previously has been found to contain large amounts of CGRP mRNA (ref. 2). We report here that immunoreactive CGRP is spontaneously released by cultured trigeminal ganglion cells and that secretion is stimulated by incubation in high potassium medium in a calcium-dependent fashion. Chromatographic characterization of the secreted CGRP-like immunoreactivity (CGRP-LI) isolated only one molecular form which appears to be similar or identical to the predicted rat CGRP (1-37).

Trigeminal ganglia were obtained from adult male Sprague-Dawley rats (Holtzman, 200–220 g) and enzymatically digested into dispersed cells using a method previously described^{4,5} (see Fig. 2 legend for details). At the time of a secretion experiment, all cells appeared healthy and well differentiated. Figure 1 shows a fluorescence photomicrograph of a cultured trigeminal ganglion cell stained using an antiserum against a synthetic analogue of CGRP, [Tyr²³]CGRP(23-37). In culture dishes prepared as described in Fig. 1 legend, a small percentage (<1%) of cells were reliably stained using a conventional indirect immunofluorescence method⁶. Cells stained for CGRP-LI were typically medium in size, measuring 18–22 μm in diameter, round or polygonal in shape, and gave rise to 4–7 processes that branched extensively in the area immediately surrounding the cell body. Cells stained in culture for CGRP-LI were thus similar in size and shape to trigeminal ganglion cells stained *in situ*², although extensive local plexuses of neuronal processes comparable to those described here could not be resolved in sections through intact ganglia.

Basal release of CGRP-LI from trigeminal ganglion cells in dispersed culture was readily measurable using our radioimmunoassay. Typically, 10–30 fmol of CGRP-LI were released per dish during a 60-min incubation period. Depolarization of cells by incubation for 60 min in high potassium (59 mM) medium typically elicited the release of 90–250 fmol of CGRP-LI per dish (~30% of total cellular content of CGRP-LI), a 7–11-fold increase over basal levels. When calcium ion was removed from the high potassium medium, the release of

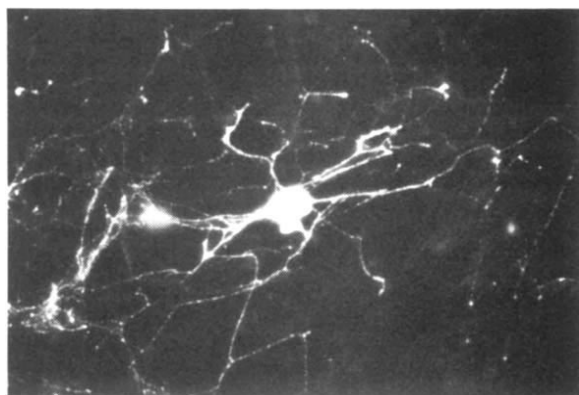


Fig. 1 Fluorescence photomicrograph of a cultured trigeminal ganglion cell. After 4–6 days in culture, dishes ($n=25$, from different preparations over a period of 2 months) were fixed for 15 min in 4% paraformaldehyde and 0.5% glutaraldehyde in borate buffer at pH 9.5. Indirect fluorescence histochemistry was then done as described in detail elsewhere⁶, using an antiserum raised against a synthetic analogue of CGRP, [Tyr²³]CGRP(23-37), conjugated to human α -globulin and used at a dilution of 1:2,000. This serum was preabsorbed with human α -globulin (7.5 $\mu\text{g ml}^{-1}$). Each of the stained processes shown in the photomicrograph could be traced to the cell body at the centre. Staining of trigeminal ganglion cells was completely blocked by the addition of synthetic [Tyr²³]CGRP(23-37) or CGRP(1-37) (1 mg ml^{-1}); addition of synthetic substance P, somatostatin(1-14), or human calcitonin at the same concentration did not influence staining.

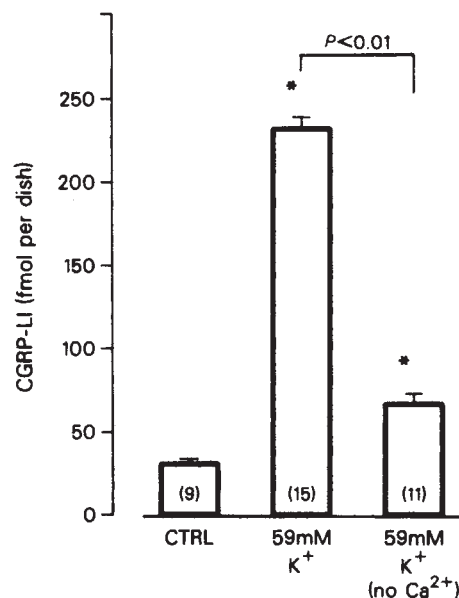


Fig. 2 Basal (CTRL) and stimulated (59 mM K⁺) release of CGRP-like immunoreactivity (CGRP-LI) from trigeminal ganglion cells in primary dispersed cell culture. The calcium (Ca²⁺) dependency of the 59 mM K⁺-induced release of CGRP-LI is also shown (59 mM K⁺, no Ca²⁺).

Methods: One hundred pairs of trigeminal ganglia were collected and dissociated enzymatically for 150 min in sterile siliconized spinner flasks using collagenase II (Worthington)^{4,5}. DNAase II (3.2 $\times 10^3$ Kunitz units; Sigma) was added at 40, 95 and 130 min. Following dispersion of the tissue, cells were washed, resuspended in HEPES-buffered Dulbecco's modified Eagle's medium (HDMEM) containing 10% fetal calf serum (FCS) and plated at a density of 5×10^6 cells per 60 mm tissue culture dish (Falcon 3002, previously coated with poly-D-lysine at 20 $\mu\text{g ml}^{-1}$)—this is equivalent to four trigeminal ganglia per dish. The culture medium was changed 3 days after plating to HDMEM + 5% FCS and secretion experiments were performed the following day. On the day of an experiment the culture medium was aspirated off the dishes and the cells were washed three times with 2.0 ml HEPES-Krebs-Ringer's-bicarbonate glucose solution (HKRBG)⁴. Cells were then equilibrated in 1.0 ml HKRBG for 1 h, followed by a 1 h experimental period in HKRBG with or without 59 mM K⁺. Finally, cells were exposed to high K⁺ (59 mM) HKRBG for 1 h to validate responsiveness. Appearance of cells following a secretion experiment was unremarkable. At the end of each incubation, the media were collected and placed in glass tubes at 4 °C, heated for 5 min at 90 °C, chilled and assayed within 12 h for CGRP-LI. Synthetic CGRP(1-37) and a C-terminal analogue, [Tyr²³]CGRP(23-37), were prepared on a *p*-methylbenzhydrylamine resin as described previously⁷ and purified using preparative scale reverse-phase HPLC⁸. Peptide purity was >95% as determined by amino acid analysis and analytical reverse-phase HPLC. Antisera were raised in New Zealand rabbits to a synthetic C-terminal analogue of CGRP, which was first coupled to human α -globulin via glutaraldehyde. The final dilution of antiserum was 1:85,000–100,000. Radiolabelled ligand ([¹²⁵I-Tyr²³]CGRP(23-37)) was prepared by the chloramine-T method and purified by reverse-phase HPLC on a Vydac C-18 column. Standard curves were constructed using CGRP(1-37). Antibody complexes were precipitated with Pansorbin (*Staphylococcus aureus* protein A; Calbiochem). The minimum detectable dose was 2–4 pg CGRP(1-37) per assay tube and the 50% effective dose (ED₅₀) of the radioimmunoassay standard curve was in the range 45–55 pg CGRP(1-37). None of the media used in our cell culture system or any of the peptides tested exhibited any cross-reactivity in the radioimmunoassay. Statistical comparisons (multiple range tests of Dunnett and Duncan) analysed the responses of dishes to test substances or control (HKRBG) during the experimental period. Numbers in parentheses indicate the number of dishes per treatment group. * $P < 0.01$.

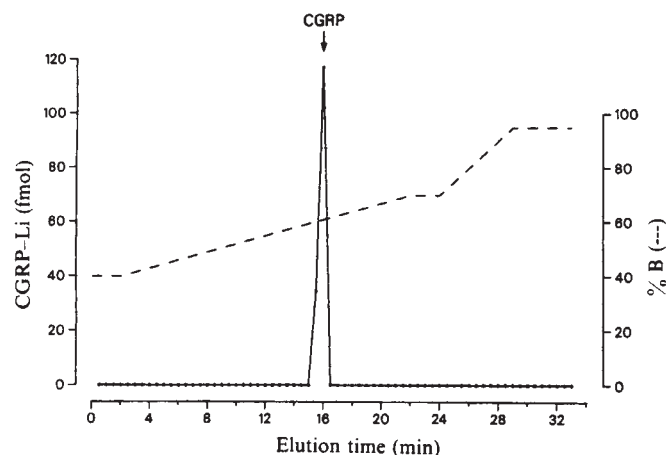


Fig. 3 Reverse-phase HPLC of pooled gel filtration chromatography samples taken from the zone containing >90% of the recoverable CGRP-LI (see text). Solvents for reverse-phase HPLC were 0.1% trifluoroacetic acid (TFA) in deionized water (solution A) and 0.1% TFA in a mixture of 60% acetonitrile/40% deionized water (solution B). Lyophilized sample or synthetic CGRP(1–37) was dissolved in 1.5 ml of solution A and 0.5–1.5 ml was applied to a Vydac C-18 column (0.46 × 25 cm, particle size 5 μ m, pore size 330 Å). Chromatograms were run for 33 min at 20–25 °C in conditions of increasing amounts of solution B (see diagram) using an Altex gradient liquid chromatograph Model 332. Flow rate was 1.5 ml min⁻¹ and fractions were collected every 0.5 min into glass tubes containing 5 μ l of 10% bovine serum albumin (BSA) in H₂O. Fractions were lyophilized, reconstituted in assay buffer, neutralized with 2 M sodium hydroxide and assayed for CGRP-LI. Blank runs of injections of solution A were performed before each application of sample or synthetic CGRP(1–37). No CGRP-LI was detected in any of these collected fractions. Recovery of CGRP-LI from samples or synthetic CGRP(1–37) across the HPLC column was ~70%. When basal or 59 mM K⁺ medium was taken directly for reverse-phase HPLC, using a Vydac or a Waters C-18 column (0.46 × 30 cm, particle size 10 μ m, pore size 120 Å), the media from 50 or 25 dishes was first pooled into an equal volume of 2 M acetic acid at 4 °C containing 12.5 mM EDTA. The acidified medium was then heated at 90 °C for 5 min, cooled to 4 °C and then partially purified over Bond Elut (Analytichem) disposable C-18 cartridges to remove large (>20,000 molecular weight) proteins. Samples were then prepared and applied to the columns as described above. The arrow on the diagram indicates the elution time of synthetic rat CGRP(1–37).

CGRP-LI was attenuated (Fig. 2). Secretion of CGRP-LI by cultured trigeminal ganglion cells resembles calcium-dependent, high potassium-induced secretion of other peptides in different *in vitro* model systems^{4,5}. Returning cells that had been stimulated with 59 mM potassium to culture medium for 18–24 h and repeating the experiment the next day demonstrated that cells could typically release significant amounts (70–100%) of CGRP-LI compared with the previous day.

Gel filtration chromatography of CGRP-LI released from trigeminal ganglion cell cultures depolarized with 59 mM potassium medium demonstrated that more than 90% of the total CGRP-LI recovered from the column eluted in the K_d range 0.28–0.41, with the peak at a K_d of 0.33. Synthetic CGRP(1–37) applied to the same column eluted in an almost identical manner (>90% CGRP-LI in K_d range 0.33–0.42, K_d peak at 0.38). In both cases only one peak of immunoreactivity was observed. No calcitonin-like immunoreactivity was detected in the column eluant or in media from cells depolarized with 59 mM potassium. In other experiments, we combined the fractions containing the CGRP-LI peak from gel filtration of medium from high potassium-stimulated cells. The pooled lyophilisate was then subjected to reverse-phase HPLC and only one peak of CGRP-LI was observed, which co-eluted with synthetic CGRP(1–37) (Fig. 3). Media from unstimulated (basal) and stimulated (59 mM potassium) cultured cells were also directly subjected to reverse-phase HPLC (following the

procedures outlined in Fig. 3) and an identical profile was observed. Together, these data provide strong evidence that only one molecular form of CGRP-LI is released from our cultured trigeminal ganglion cells and that this is very similar, if not identical, to synthetic rat CGRP(1–37).

In summary, our data provide the first direct evidence of secretion of CGRP-LI from nervous tissue and support the possibility that CGRP may have a role as an extracellular modulator. Secretion appears to depend on the availability of extracellular calcium, as is expected in secretory systems of established physiological significance. Only one molecular form of CGRP-LI, apparently identical to the predicted rat CGRP(1–37), is released. Our observations agree with a previous report describing high levels of CGRP immunoreactivity and CGRP mRNA in trigeminal ganglia² and with studies suggesting a tissue specificity in the generation of mRNAs encoding calcitonin or CGRP^{1,2}. Furthermore, the data support the possibility that alternative RNA processing mechanisms are a biologically significant means of increasing the diversity of extracellular regulatory molecules.

This research was supported by NIH grant AM-26741 and a grant from The Keck Foundation. This research was conducted in part by the Clayton Foundation—California Division. P.E.S. and W.W.V. are Clayton Foundation Investigators. R.T.M. is a University of Melbourne Research Fellow. R.A.P. is the recipient of a Medical Scientist Training Program Award PHS GM07198. The support, advice and assistance of Drs T. Bruhn, H. Seifert, M. G. Rosenfeld, R. Evans, L. Swanson, L. Bilezikian and M. Chen, and M. Boyle, D. Chin and R. McClintock are gratefully appreciated.

Received 14 November 1983; accepted 1 February 1984.

1. Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S. & Evans, R. M. *Nature* **298**, 240–244 (1982).
2. Rosenfeld, M. G. *et al.* *Nature* **304**, 129–135 (1983).
3. Fisher, L. A. *et al.* *Nature* **305**, 534–536 (1983).
4. Peterfreund, R. A. & Vale, W. W. *Brain Res.* **239**, 463–477 (1982).
5. Shoemaker, W. J., Peterfreund, R. A. & Vale, W. W. *Meth. Enzym.* **103**, 347–362 (1983).
6. Sawchenko, P. E. & Swanson, L. W. *Brain Res.* **210**, 31–51 (1981).
7. Marki, W., Spiess, J., Tache, Y., Brown, M. & Rivier, J. E. *J. Am. chem. Soc.* **103**, 3178–3185 (1981).
8. Rivier, J., McClintock, R., Galyean, R. & Anderson, H. *J. Chromatogr.* (in the press).

Recombinant nontoxinogenic *Vibrio cholerae* strains as attenuated cholera vaccine candidates

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An ideal vaccine does not yet exist to prevent cholera, a significant health problem in many less developed countries. *Vibrio cholerae*, the agent of epidemic and endemic cholera, colonizes the small bowel and secretes a potent enterotoxin that consists of a single A subunit, which stimulates adenylate cyclase activity, and five identical B subunits which bind to the ganglioside GM₁ receptor of intestinal mucosal cells¹. Previous studies in man indicate that toxoid-derived antitoxic immunity by itself is insufficient to provide effective, long-lasting protection against cholera^{2–4}. Using recombinant DNA techniques we have now constructed a live, attenuated *V. cholerae* strain by deleting genes encoding the enterotoxin. Restriction enzyme fragments encoding cholera toxin were deleted *in vitro* from cloned *vibrio* chromosomal DNA and the resulting mutations introduced into the chromosome of a *vibrio* strain of proven immunogenicity. Recently, Mekalanos and coworkers⁵ have reported attenuated *V. cholerae* strains constructed by similar methods. It appears that recombinant DNA techniques offer a promising approach to the development of effective cholera vaccines.

Experimental cholera studies in community volunteers at the Center for Vaccine Development, University of Maryland, have

Fig. 1 Deletion of cholera toxin genes from cloned *V. cholerae* chromosomal DNA. **a**, Deletion of toxin genes. A 4 kb *Pst*I–*Bgl*II fragment of the recombinant plasmid pJBK16 (ref. 12) contains genes encoding cholera toxin flanked by *Acc*I sites with a third *Acc*I site in the B subunit structural gene. The two *Acc*I fragments were removed and a fragment from pREG153 encoding β -lactamase was cloned in their place. The heavier lines indicate vibrio DNA sequences. **b**, Addition of flanking *V. cholerae* chromosomal DNA sequences to toxin gene mutation. Plasmid pJBK33 consists of a 20 kb chromosomal *Hind*III DNA fragment from *V. cholerae* N16991 cloned into pBR325 (pJBK33 contains 11 *Acc*I sites and so was unable to be as easily mutated as pJBK16). All but one *Pst*I site was removed by cloning a 19 kb *Eco*RI–*Sal*I fragment into pJBK40 to yield pJBK44. pJBK44 contains a 4.0 kb *Pst*I–*Bgl*II fragment bearing toxin genes as in pJBK16 flanked by approximately 7.0 and 7.5 kb of *V. cholerae* N16961 chromosomal DNA. Partial digestion with *Bgl*II yielded a population of linear molecules each digested at one of four *Bgl*II sites. These linear molecules were then digested to completion with *Pst*I and a 21 kb fragment containing all the sequences of pJBK44 except for the 4.0 kb *Pst*I–*Bgl*II toxin fragment was isolated. Plasmid pJBK21 DNA was partially digested with *Pst*I and then cut to completion with *Bgl*II to generate a 3.8 kb *Pst*I–*Bgl*II fragment containing the β -lactamase genes cloned in place of the cholera toxin genes. The purified fragments from pJBK44 and pJBK21 were then ligated to yield pJBK54, which contains a 19 kb *Hind*III–*Sal*I fragment of *V. cholerae* chromosomal DNA with β -lactamase cloned in place of the deleted cholera toxin genes.

Methods: **a**, pJBK16 (Tp^r) was digested to completion with the restriction endonuclease *Acc*I (BRL) and the resulting fragments separated by gel electrophoresis through 0.7% agarose. The largest fragment contained the plasmid vector and DNA sequences flanking, but not including the genes encoding cholera toxin. This fragment was electroeluted from the gel and extracted with phenol and ether. The ends were made blunt ended by filling in with the Klenow fragment of DNA polymerase I (BRL) according to the manufacturer's instructions. A fragment containing genes encoding β -lactamase and therefore resistance to ampicillin was obtained from plasmid pREG153 (received from R. E. Gill) by digesting with *Bam*HI and *Eco*RI (BRL) and purifying by agarose gel electrophoresis. The ends of this fragment were filled in with Klenow fragment, mixed with the purified, blunt-ended fragment from pJBK16 and ligated overnight using T4 DNA ligase (BRL) at 15 °C. The ligation mixture was then transformed into *Escherichia coli* HB101 and plated on Mueller Hinton agar (Difco) containing 50 μ g ml⁻¹ trimethoprim and 100 μ g ml⁻¹ ampicillin. Plasmids from the transformants were extracted by the method of Birnboim and Doly¹⁷ and digested by a variety of restriction enzymes, including *Pst*I, *Bgl*II, *Eco*RI and *Bam*HI. One clone which gave the expected restriction pattern was designated pJBK21. **b**, pJBK33 DNA was digested to completion with *Eco*RI and *Sal*I (BRL) and mixed with pJBK40 DNA similarly digested with *Eco*RI and *Sal*I. pJBK40 is an ampicillin sensitive derivative of pBR325 which lacks a *Pst*I site. It was constructed by digesting pBR325 with *Pst*I and transforming linear molecules into *E. coli* strain HB101. Recircularization within the *E. coli* cell resulted in loss of Ap resistance and the *Pst*I site. Digested pJBK33 (Ap^r, Cm^r) and pJBK40 (Cm^r, Tc^r) DNA was ligated overnight at 15 °C with T4 ligase and transformed into HB101. Plasmid content of Cm^r, Tc^r, Ap^r colonies was analysed and one clone which gave the expected restriction pattern with only a single *Pst*I site was designated pJBK44. Linear molecules of pJBK44 in which only one of the four *Bgl*II sites was cut were obtained by digesting pJBK44 with *Bgl*II enzyme in the presence of 0.1 mg ml⁻¹ ethidium bromide¹⁸. After linearization, the ethidium bromide was removed by extraction with water saturated *n*-butanol and the DNA precipitated with ethanol. The *Bgl*II linears were then digested to completion with *Pst*I and the resulting fragments separated by gel electrophoresis through 0.3% agarose. The separated fragments were eluted, purified and analysed by digestion with *Bgl*II to identify the desired fragment, that is, a 21 kb fragment with all the DNA of pJBK44 except for the 4 kb *Pst*I–*Bgl*II fragment containing the toxin genes. Plasmid pJBK21 was treated similarly by linearization with *Pst*I in ethidium bromide followed by *Bgl*II digestion and separation by agarose gel electrophoresis. The desired fragment, a 3.8 kb *Bgl*II–*Pst*I fragment containing β -lactamase with an internal *Pst*I site, was mixed with the purified fragment from pJBK44, ligated and transformed into HB101. Plasmid DNA from Cm^r, Ap^r transformants was analysed and one clone which gave the expected restriction endonuclease pattern was designated pJBK54. This plasmid was identical to pJBK44 except for the substitution of the 3.8 kb *Pst*I–*Bgl*II fragment encoding β -lactamase from pJBK21 for the 4.0 kb *Pst*I–*Bgl*II fragment encoding cholera toxin from pJBK33.

Fig. 2 Introduction of cholera toxin gene deletion into the chromosome of enterotoxigenic *V. cholerae* N16961. **a**, The plasmid pJBK55 was constructed by digesting pJBK54 (Ap^r, Cm^r) (Fig. 1b) with *Eco*RI and *Sal*I, ligating and selecting Ap^r, Tc^r, Cm^r transformants. pJBK55 was mobilized from *E. coli* HB101 into *V. cholerae* N16961 with the aid of a conjugative 'helper' plasmid pRK2013 (ref. 15). **b**, Selection of *V. cholerae* cells in which the toxin deletion mutation had recombined into the chromosome. Plasmid pR702 (Su^r, Sm^r, Tc^r, Km^r) (ref. 16) belongs to the Inc P group and was mated into *V. cholerae* N16961 (pJBK55). PB^r, Ap^r and Su^r colonies were selected, screened for Cm sensitivity and tested for toxinogenicity by GM₁ enzyme-linked immunosorbent assay (ELISA). **c**, One nontoxigenic mutant, designated *V. cholerae* JBK56, was selected and confirmed as nontoxigenic by GM₁ ELISA, Y-1 adrenal cell assay and DNA hybridization using a labelled cholera toxin gene probe.

Methods: Restriction enzyme digestion, ligation and plasmid extraction methods are as given in the legend for Fig. 1. **a**, Conjugal mating between *E. coli* HB101 (pJBK55) and *V. cholerae* N16961 was carried out by mixing 1 ml of mid-log cultures of donor and recipient grown statically at 37 °C in L-broth. The mixed cultures were filtered through 0.4 μ m filters (Nuclepore) which were then placed on L-agar plates for approximately 4 h at 37 °C. At the end of the mating period, bacteria on the filter were resuspended in 1 ml L-broth and plated on L-agar containing 100 μ g ml⁻¹ ampicillin and 50 U per ml polymyxin B (Aerosporin; Burroughs Wellcome). (*V. cholerae* El Tor, unlike *E. coli*, is naturally resistant to 50 U per ml polymyxin B.) Plasmid content of Ap^r *V. cholerae* transconjugants was examined to confirm the presence of pJBK55. **b**, Conjugal mating between *E. coli* J53 (pR702) and *V. cholerae* N16961 (pJBK55) was performed as described above with selection on L-agar with 100 μ g ml⁻¹ ampicillin and 100 μ g ml⁻¹ sulphonamide. **c**, Toxinogenicity testing by the Y-1 adrenal cell assay and GM₁ ELISA was performed as previously described^{19,20}. DNA hybridization studies were performed using a colony hybridization technique²¹ and labelled cholera toxin gene sequences. A cholera toxin gene probe was prepared by labelling a 1,200 base pair (bp) *Acc*I fragment from pCVD002 (ref. 13) by nick translation²² using (³²P-dATP (800 Ci mmol⁻¹; NEN) and DNA polymerase I (NEN). Approximately 10⁶ c.p.m. of labelled DNA was hybridized to the nitrocellulose filter in a solution consisting of 50% formamide, 5 $\times$ SSC (SSC: 0.15 M NaCl plus 0.015 M sodium citrate), 1 $\times$ Denhardt solution (0.02% Ficoll, molecular weight (MW) 400,000; 0.02% polyvinyl pyrrolidone, MW 360,000; 0.02% bovine serum albumin), 0.1% SDS and 1 mM EDTA at 37 °C for 18 h. After washing in 5 $\times$ SSC–0.1% SDS at 65 °C for 1 h, the blots were dried and exposed to Kodak XR-5 film for autoradiography.

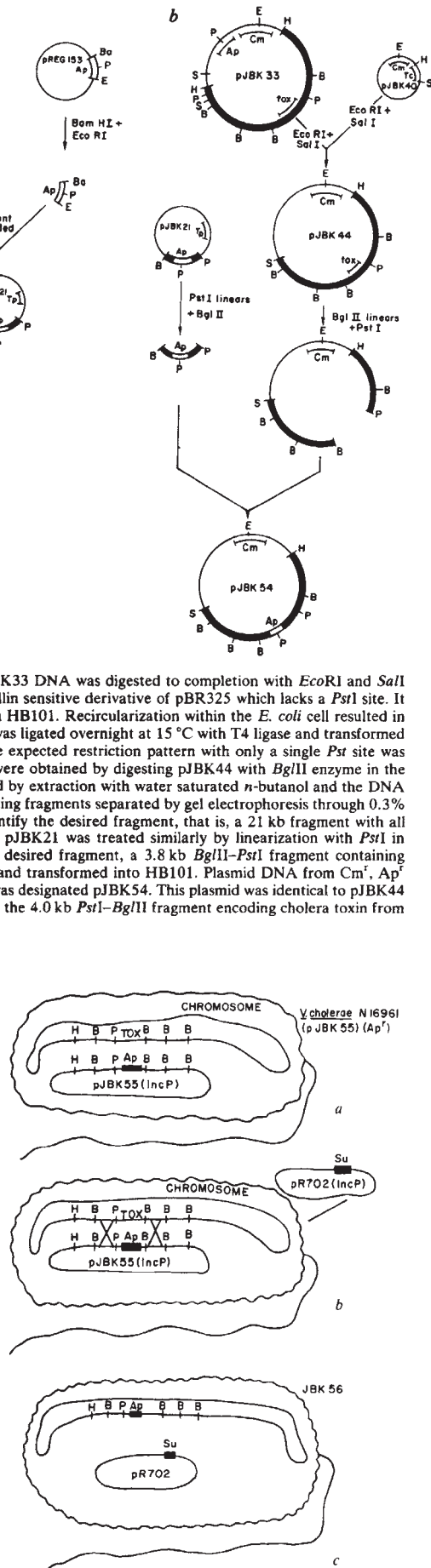
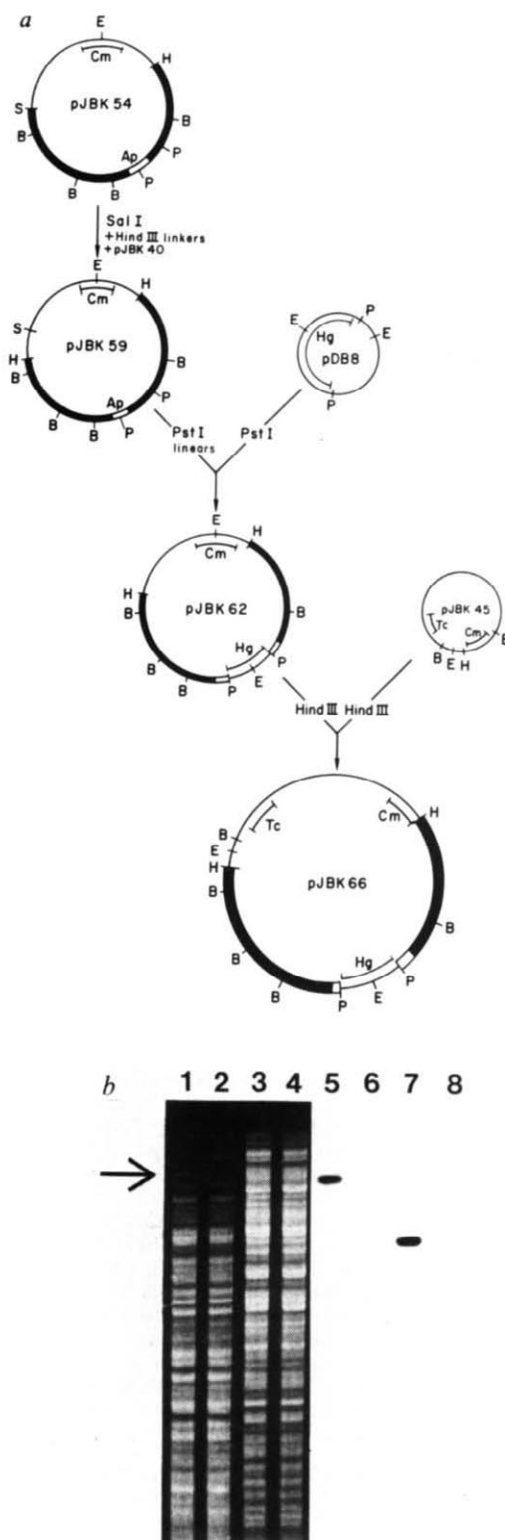


Fig. 3 Construction of mercury resistant, ampicillin sensitive non-toxinogenic *V. cholerae*. **a**, Construction of *V. cholerae* JBK70 (Hg^r). A second *Hind*III restriction site was added to pJBK54 by digesting pJBK54 with *Sal*I, filling in the ends with Klenow fragment and ligating *Hind*III linkers (BRL) to the filled in *Sal*I site. The ligated mixture was digested with *Hind*III and the large fragment containing the mutated vibrio DNA and the β -lactamase was purified by agarose gel electrophoresis. The purified fragment was ligated to *Hind*III digested pJBK40, transformed into *E. coli* HB101 and Ap^r, Cm^r clones were analysed for the presence of two *Hind*III sites flanking the vibrio DNA. The resulting plasmid, pJBK59, was partially digested with *Pst*I in the presence of ethidium bromide to yield linear molecules. These were mixed with *Pst*I-digested pDB8 (received from A. Summers), which contains the mercury resistance genes from R100 on a 7.5 kb *Pst*I fragment. Overnight ligations were transformed and Cm^r, Hg^r (50 M HgCl₂), Ap^r clones were analysed yielding pJBK62. The *Hind*III fragment from pJBK62 containing vibrio DNA and the mercury resistance genes inserted into the β -lactamase *Pst*I site was purified and cloned into pJBK45 to yield pJBK66. pJBK45 is a derivative of pRK290 (ref. 15) which contains a 4 kb *Eco*RI fragment containing chloramphenicol resistance gene and a *Hind*III site from the plasmid vector pKT212 (ref. 23). This plasmid was mobilized into *V. cholerae* JBK56 which had been spontaneously cured of pR702 and the homologous recombination process repeated (Fig. 2). The resulting nontoxigenic Hg^r Ap^r *V. cholerae* strain was subsequently spontaneously cured of pR702 and was designated JBK70. **b**, Change in *V. cholerae* N16961 chromosomal restriction fragments due to homologous recombination of deleted toxin gene region shown by agarose gel electrophoresis and Southern blot analysis of restriction endonuclease fragments of *V. cholerae* strains N16961 and JBK70. Lanes 1, 5: N16961, *Hind*III; lanes 2, 6: JBK70, *Hind*III; lanes 3, 7: N16961, *Pst*I; lanes 4, 8: JBK70, *Pst*I. The nontoxigenic derivative, pJBK70, shows no homology with the cholera toxin probe, as shown in lanes 6 and 8. In lane 2, the total chromosomal digest of JBK70 appears unaltered from the parent strain (lane 1) except for the change in molecular weight of the largest *Hind*III fragment of N16961, which contains the toxin gene (indicated by arrow). As would be expected, the largest *Hind*III fragment shows an increase of approximately 7 kb due to the insertion of the mercury resistance gene (lane 2).

Methods: Restriction enzyme digestion, ligation and plasmid extraction methods are as given in the legend for Fig. 1. Chromosomal DNA was extracted from toxigenic *V. cholerae* strain N16961 and the nontoxigenic mutant JBK70 by the method of Brenner *et al.*²⁴. Approximately 1 μ g of DNA was digested with restriction endonuclease *Hind*III or *Pst*I (BRL) and restriction fragments were separated on a 0.7% agarose gel, transferred to nitrocellulose paper by the method of Southern²⁵ and hybridized to a ³²P-labelled cholera toxin gene probe as described in the legend to Fig. 2.

demonstrated that, as in nature, protective immunity follows infection with pathogenic *V. cholerae* and these studies have identified toxigenic, immunogenic strains for attenuation as candidate cholera vaccines⁶⁻¹⁰. One such strain, N16961 (El Tor Inaba)⁸, extensively studied in volunteers, was attenuated by specific deletions of restriction fragments containing the genes encoding cholera toxin. The fragments encoding enterotoxin were deleted *in vitro* from the recombinant plasmid pJBK16^{11,12}, and replaced with a fragment encoding β -lactamase and therefore resistance to ampicillin (Fig. 1a). Additional flanking vibrio chromosomal DNA was then added to ensure the homologous recombination of this deletion into the chromosome (Fig. 1b). The resulting plasmid, pJBK54, contained the β -lactamase in place of the toxin genes flanked by approximately 7 and 9 kilobases (kb) of vibrio DNA.

The construct was then introduced into the chromosome of N16961, by site-directed mutagenesis¹³. The mutated vibrio DNA in pJBK54 was cloned into the incompatibility group P plasmid pRK290¹⁴ to produce pJBK55 which was then introduced into *V. cholerae* N16961 (Fig. 2a). In a small percentage of cells, the plasmid vibrio DNA sequences flanking the β -lactamase genes recombined with the homologous chromosomal sequences flanking the toxin genes. Homologous cross-over occurred, resulting in displacement of the toxin genes by the



β -lactamase genes (Fig. 2b). This event was detected in a population of *V. cholerae* containing pJBK55 by introduction of a second P-group plasmid, pR702 (ref. 15), which is incompatible, that is, cannot be stably maintained with derivatives of pRK290. Resulting ampicillin and sulphonamide resistant cells represent those cells in which pR702 (Su^r, Sm^r, Tc^r, Km^r) is stably maintained extrachromosomally, the ampicillin resistance is integrated into the chromosome and the toxin genes and the pRK290 replicon are lost (Fig. 2c). One such mutant, JBK56, was found to lack the genes encoding cholera toxin. Because of possible clinical objections to a vaccine resistant to a widely used antibiotic, a second mutant, JBK70, was constructed which substitutes mercury resistance for ampicillin resistance (Fig. 3).

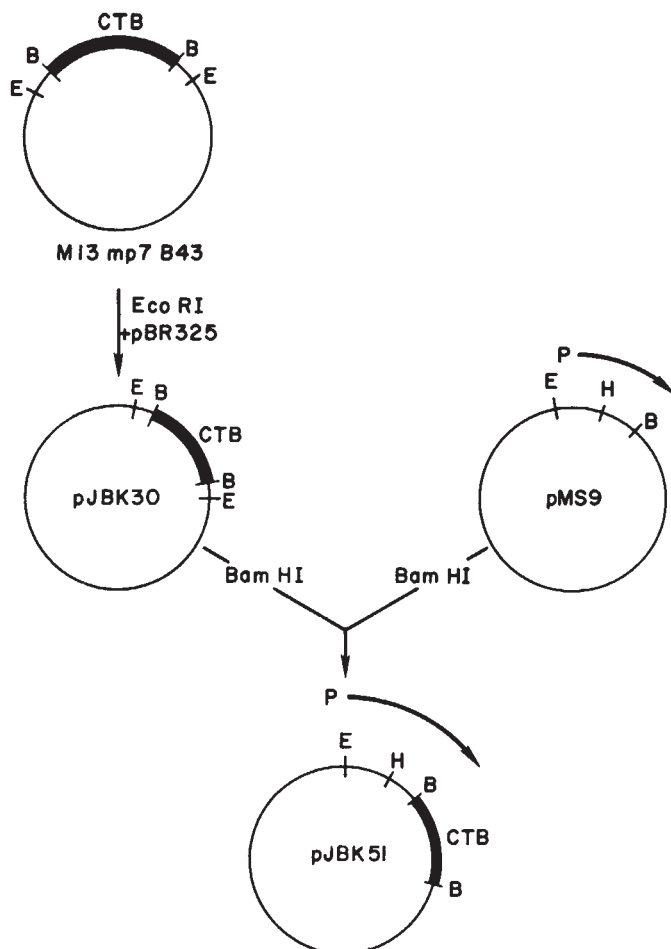


Fig. 4 Construction of *V. cholerae* JBK70 (pJBK51), an A⁻B⁺ derivative. During DNA sequencing studies of cholera toxin¹³ a 1.3 kb *Hpa*II fragment containing the A₂ and B, but not the A₁ subunit genes, was cloned into the M13 mp7 B43. The placement in the M13 vector positioned *Bam*HI and *Eco*RI sites adjacent to this fragment which was subsequently cloned into pBR325, yielding pJBK30. The *Bam*HI fragment from pJBK30 was then cloned into pMS9, which contains the *trp* promoter from *Serratia marcescens*. This vector consists of a pBR32R vector plus 94 bp of *S. marcescens* DNA containing the promoter, but no attenuator region or ribosome binding site (ref. 26 and C. Yanofsky, personal communication). A putative ribosome binding site for the cholera toxin B subunit is found in the structural gene for the A₂ subunit¹³ and when the *Bam*HI fragment from pJBK30 was cloned into the *Bam*HI site of pMS9, ~50% of the resulting clones produced B subunit (as measured by GM₁ ELISA), reflecting the orientation of the insert. The resulting plasmid, pJBK51, was mobilized into *V. cholerae* JBK70 by pRK2013 to produce an attenuated *V. cholerae* strain producing B subunit only.

Volunteer studies indicate that an antibacterial response is essential for immunity⁴, so immunization with the non-toxinogenic strain may be sufficient for protection against pathogenic toxinogenic *V. cholerae*. Inhibition of the initial colonization of the vibrios along the mucosa of the small bowel is apparently the key immune mechanism in the protection that follows natural infection. Nevertheless, as antibodies to cholera toxin work synergistically in animal models to enhance immunity, a plasmid has been constructed which produces only the nontoxic B subunit (Fig. 4). This plasmid has been introduced into *V. cholerae* JBK70, after which it was found to produce B antigen but not holotoxin. A previously studied A⁻B⁺ *V. cholerae* strain, Texas Star-SR, attenuated by multiple rounds of nitrosoguanidine mutagenesis¹⁶, stimulated infrequent and meagre antitoxin responses that appeared to be unrelated to successful protection against challenge with pathogenic *V. cholerae*⁹. Attenuated vaccine strains derived by such methods,

however, suffer from a number of disadvantages: (1) the precise genetic mutation is unknown so the theoretical risk of reversion to toxinogenicity remains; (2) nitrosoguanidine may induce other unrecognized mutations affecting other antigens that contribute to immunity. The method we have described is free of these disadvantages as it eliminates the possibility of reversion to toxinogenicity, due to the complete deletion of the toxin genes, while it leaves unaffected all other antigens important for immunity. Clinical studies are underway at the Center for Vaccine Development to assess the safety and efficacy in man of the attenuated *V. cholerae* strain JBK70.

We thank Anne O. Summers, Charles Yanofsky, Walt Dallas and Ron Gill for providing plasmids, John P. Craig for rabbit skin permeability factor assays, Sue Trinker, Jane Michalski and Yu Lim for technical help and Jorge Flores for helpful discussion and criticisms. This work was supported by NIH grant R22AI19716 and contract N01AI12666.

Received 22 August 1983; accepted 19 January 1984.

- Holmgren, J. *Nature* **292**, 413-417 (1981).
- Curlin, G., Levine, R., Azia, K. M. A., Mizanur Rahman, A. C. M. & Verway, W. F. *Proc. 11th Joint US-Japan Cholera Conf.*, New Orleans, 314-329 (Dept of Health, Education and Welfare, Bethesda, 1976).
- Noriki, H. *Proc. 12th Joint US-Japan Cholera Conf.*, Sapporo, 302-310 (Fuji, Tokyo, 1977).
- Levine, M. M. *et al. Trans. R. Soc. trop. Med. Hyg.* **73**, 3-9 (1979).
- Mekalanos, J. J. *et al. Nature* **306**, 551-557 (1983).
- Levine, M. M. in *Cholera and Related Diarrhoeas: 43rd Nobel Symp.*, Stockholm, 195-203 (Karger, Basel, 1980).
- Levine, M. M. *et al. J. infect. Dis.* **143**, 818-820 (1981).
- Levine, M. M. *et al. in Acute Enteric Infections in Children. New Prospects for Treatment and Prevention* (eds Holme, T., Holmgren, J., Merson, M. H. & Mollby, R.) 449-459 (Elsevier, Amsterdam, 1981).
- Levine, M. M. *et al. Infect. Immun.* **43**, 512-522 (1984).
- Glass, R. I. *et al. Am. J. Epidemiol.* **116**, 959-970 (1982).
- Kaper, J. B. & Levine, M. M. *Lancet* **ii**, 1162-1163 (1981).
- Lockman, H. & Kaper, J. B. *J. biol. Chem.* **258**, 13722-13726 (1983).
- Ruvkun, G. B. & Ausubel, F. M. *Nature* **289**, 85-88 (1981).
- Ditta, G., Stanfield, S., Corbin, D. & Helinski, D. R. *Proc. natn. Acad. Sci. U.S.A.* **77**, 7347-7351 (1980).
- Jacob, A. E. *et al. in DNA Insertion Elements, Plasmids, and Episomes* (eds Bukhari, A. I., Shapiro, J. A. & Adhya, S. L.) 607-638 (Cold Spring Harbor Laboratory, New York, 1977).
- Honda, T. & Finkelstein, R. A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 2052-2056 (1979).
- Birnboim, H. C. & Doly, J. *Nucleic Acids Res.* **7**, 1513-1523 (1979).
- Parker, R. C., Watson, R. M. & Vinograd, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 851-855 (1977).
- Sack, D. A. & Sack, R. B. *Infect. Immun.* **11**, 334-336 (1975).
- Sack, D. A., Huda, S., Neogi, P. K. B., Daniel, R. R. & Spira, W. M. *J. clin. Microbiol.* **11**, 35-40 (1980).
- Moseley, S. L. *et al. J. infect. Dis.* **142**, 892-898 (1980).
- Maniatis, T., Jeffrey, A. & Kleid, A. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184-1188 (1975).
- Bagdasarian, M., Bagdasarian, M. M., Coleman, S. & Timmis, K. N. *Plasmids of Medical, Environmental and Commercial Importance* (eds Timmis, K. N. & Puhler, A.) 411-422 (Elsevier, Amsterdam, 1979).
- Brenner, D. J., Fanning, G. R., Johnson, K. E., Citarella, R. V., & Falkow, S. *J. Bact.* **98**, 637-650 (1969).
- Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
- Miozzari, G. F. & Yanofsky, C. *Nature* **276**, 684-689 (1978).

Scrapie infectious agent is virus-like in size and susceptibility to inactivation

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The virions of all known viruses are composed of small amounts of genomic nucleic acid enveloped by proteins and other macromolecules. The aetiological agents of scrapie disease and the other subacute spongiform virus encephalopathies (SSVE), a group of slow, fatal degenerative diseases of the central nervous system, are, based on their resistance to sterilization and on indirect measurements suggesting subviral size, thought to have non-viral structures (see refs 1-3 for reviews). The kinetic studies reported here demonstrate that scrapie's resistance to many inactivants is limited to small subpopulations of the total infectivity, the majority population being highly sensitive to inactivation. Moreover, control inactivations of conven-

tional viruses provide examples of both scrapie-like resistant subpopulations and complete insensitivity to virucidal agents, especially when those viruses, like scrapie, are suspended in hamster brain homogenate. Virus controls further establish that the ability of the scrapie agent to penetrate dilute agarose-acrylamide electrophoretic gels^{4,5} is shared by conventional viruses. Direct comparison of scrapie's resistance to ionizing radiation with the resistances of other viruses places scrapie with the smaller viruses, as opposed to requiring a subviral size as claimed⁶⁻¹⁰.

Scrapie infectivity is refractory to sterilization by many potent virucidal agents. However, since sterilization requires total destruction of infectivity, it measures only the most resistant subpopulation of a virus. In contrast, structural inferences must be deduced from the behaviour of the majority population of a virus as revealed in the initial rate of inactivation. All methods of quantitation of scrapie titres rely ultimately on end point dilution titration in animals, which, as typically used, can distinguish, at best, a $10^{0.5}$ difference in titre between two samples¹¹. It is thus difficult to establish sensitivity of the scrapie agent to any inactivant that kills less than 90% even though it may be very active against the majority population. To be detected, a scrapie inactivation must be sufficiently first order in character and extensive enough to permit extrapolation to time zero.

Past scrapie inactivations have usually been conducted on crude brain suspensions. To control for the effects of brain homogenate on the inactivation kinetics, highly purified bacteriophages (Φ X174, PM2, λ and fd) were added to normal hamster brain homogenate and to phosphate-buffered physiological saline pH 7.2 (PBS) without brain homogenate and subjected to inactivation procedures identical to those for scrapie.

The majority of the scrapie infectivity was rapidly inactivated by 0.525% hypochlorite (Fig. 1a) and 0.01 M sodium metaperiodate pH 3.6 (Fig. 1b), leaving resistant subpopulations of 0.1–0.01%. Resistant subpopulations were also observed in the inactivations of bacteriophages Φ X174, fd and, in the case of periodate, phage λ , though only in the presence of 10% brain

homogenate. The extent of the initial inactivation of scrapie infectivity by 2% aqueous iodine (Fig. 2a) is greater in 1% than in 10% brain homogenate, again indicating the role of brain homogenate in the apparent stability of the scrapie agent. (A better interpretation of the apparent increase in scrapie titre in the 1% brain sample is that both the 1% and 10% inactivations have plateaued within 15 min at a value approximated by the mean of the 15, 30 and 60 min points.)

The total extent of inactivation of the scrapie agent by 0.5% aqueous phenol (Fig. 2b), 3% hydrogen peroxide (Fig. 2c), or 0.1% potassium permanganate (Fig. 2d) is $\leq 90\%$ even after 60 minutes of exposure. Therefore, the initial activation rate cannot be determined by these methods. Scrapie may undergo a rapid but limited initial inactivation like that observed for phage λ in the presence of 3% hydrogen peroxide and brain homogenate (Fig. 2c), or scrapie may be only slowly inactivated. However, even slow inactivation would not necessarily constitute a unique scrapie-specific property as evidenced by the unexpected insensitivity of bacteriophage Φ X174 to aqueous phenol (Fig. 2b).

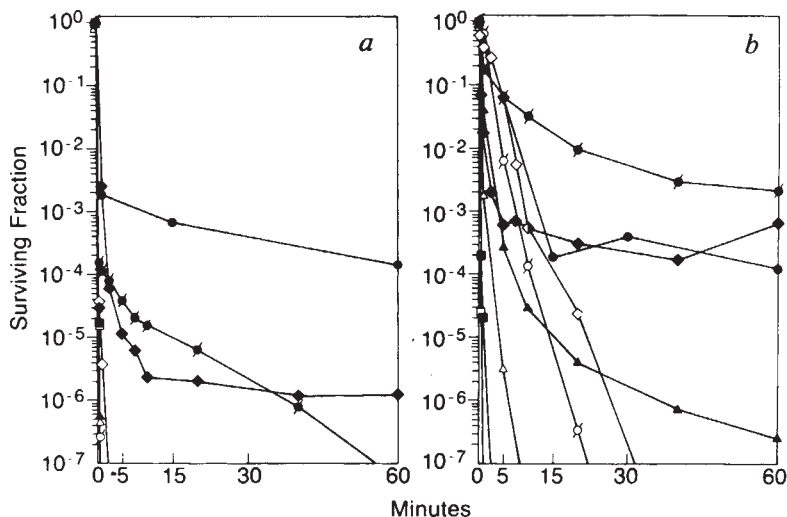
Infectivity has been recovered from scrapie- and other SSVE-infected tissue specimens stored for months in formaldehyde solution¹²⁻¹⁷. In contrast, kinetic measurement reveals that 98% of the scrapie infectivity in a 10% brain homogenate is destroyed during the first 4 h of exposure to 10% formaldehyde solution (Fig. 3). Other viruses have shown remarkable resistance to complete inactivation by formaldehyde¹⁸⁻²⁰ and experience with vaccine production methods has revealed the sensitivity of the formaldehyde inactivation to the presence of contaminating substances²¹. The scrapie inactivation may be affected in a similar manner.

It has been reported that scrapie cannot be inactivated by boiling or even by autoclaving (see ref. 22 for review). However, in virus-controlled kinetic studies in which the sample temperatures were constantly monitored by an embedded thermistor, the vast majority of the scrapie infectivity was destroyed immediately on exposure to temperatures of 100 °C or above²². Thermal resistance reposed in small subpopulations, less than

Fig. 1 Inactivation of scrapie and four control viruses by a, hypochlorite ion; b, periodate ions. Symbols: $\bullet$, scrapie; $\circ$, Φ X174am3; $\blacksquare$, $\square$, PM2; $\blacktriangle$, $\triangle$, λ ; $\blacklozenge$, $\lozenge$, fd. Closed symbols represent inactivation in 10% brain homogenate. Open symbols represent PBS.

Methods: Twenty per cent suspension of scrapie-infected (early clinical disease; hamster strain 263K obtained from R. Kimberlin^{51,52} and passaged twice in this laboratory) and normal hamster brains were prepared in PBS pH 7.4 by exhaustive sonication and low-speed centrifugation (International PR-2, infected, and Sorvall HS4, uninfected, 2,000 r.p.m., 4 °C, 15 min), discarding the pellet. The scrapie suspension used in a and in Fig. 2b–d was stored at 4 °C, used the same day and titred 6.3×10^8 ID₅₀ units ml⁻¹. That used in b, and in Figs 2a and 3 was frozen at -70 °C for 24 h, thawed and resonicated just before use and titred 1.59×10^9 ID₅₀ units ml⁻¹. The several pools of normal brain homogenate used were stored at -30 °C until just before use. Filamentous bacteriophage fd and the lipid-containing phage PM2 were obtained from the American Type Culture Collection, along with their respective hosts, *Escherichia coli* strain Hfr and *Alteromonas espejiani*. Phage λ strain Y1 and host *E. coli* N720 were obtained from Howard Nash. Phage Φ X174am3 and host *E. coli* CQ2 were obtained from Clyde Hutchison. A λ resistant mutant of *E. coli* CQ2, designated CQ2 lambda^r, was selected from a λ -infected culture of CQ2.

All phages could be titrated in the presence of one another. Purified, high-titre stocks, 10^{12} – 10^{14} plaque forming units (PFU) per ml were prepared by CsCl bandings of polyethylene glycol precipitates of low speed supernatants of naturally lysed cultures of PM2, polyethylene glycol precipitates of whole cell supernatants of productive stationary phase cultures of fd, virus pellets of low speed supernatants of chloroform lysed, lytic infections of λ and lysozyme lysates of cell concentrates of lysis-deficient Φ X174am3 grown on a non-permissive host. Small aliquots ($\leq 1\%$ of the total volume) of the phages were added to 20% normal hamster brain homogenate and PBS to give final titres approximating that of the scrapie agent, 10^9 PFU ml⁻¹. Brain homogenate alone did not inactivate the phages. Inactivations were conducted by sampling the 20% homogenate and, in the case of the phage experiments, the PBS suspension, for a time zero point. An equal volume of freshly prepared test reagent (reagent grade chemicals except a and Fig. 2b) was then added to give the final concentrations given in the figure legends. The scrapie samples were stirred continuously with magnetic stir bars in small beakers. The phage suspensions, except where noted, were continuously mixed in a Buchler vortex evaporator without evaporation. Samples were taken at the times indicated and immediately diluted serially in PBS (scrapie) or tryptone broth (phage) and inoculated into hamsters (scrapie) or onto agar petri plates with indicator bacteria (phage). Each of four weanling, female, golden Syrian hamsters (Charles River-Lakeview Hamsters) was inoculated intracerebrally in the left hemisphere with 0.05 ml at each dilution. 10–15 min were required to inoculate the complete dilution series in the case of scrapie, and, in the case of the phages, 3–8 min depending on the number of different phages being titrated. However, in every case the reaction mixture was diluted 100–1,000-fold and greater within moments of sampling, thereby effectively stopping the reaction. The inoculated hamsters were observed for 308 days (a and Figs 2b–d) or 291 days (b and Figs 2a, 3) and scored positive for scrapie only after histological confirmation of spongiform change in coded brain sections. Titres were calculated by the method of Reed and Muench⁵³. a, 0.525% (wt/vol) sodium hypochlorite, a 1/10 dilution of Rainbow Bleach (J. L. Prescott Co., Passaic); b, 0.01 M sodium metaperiodate, 0.1 M sodium acetate (pH 3.6). The periodate solution and reaction mixtures were protected from light. Due to the low pH of the periodate solution a precipitate developed which was resuspended by trituration before inoculation.



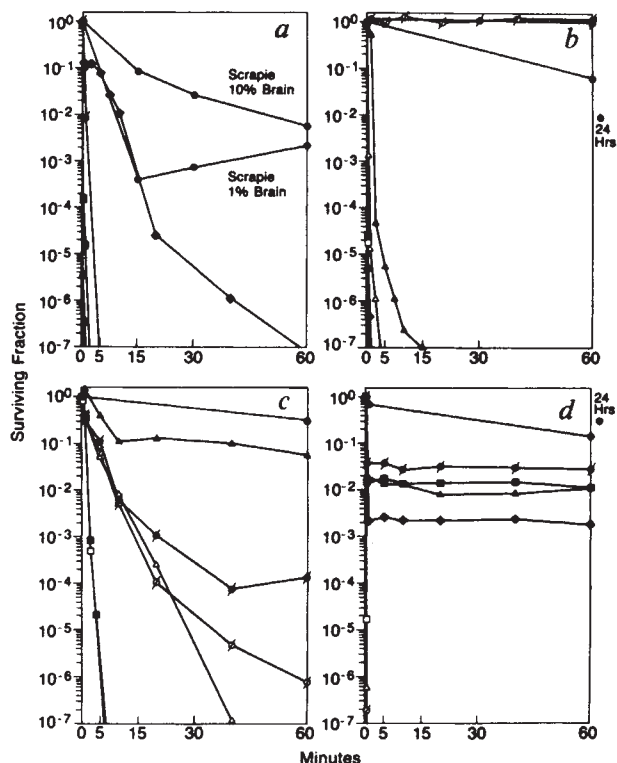


Fig. 2 Inactivation of scrapie and four control viruses by: *a*, molecular iodine; *b*, aqueous phenol; *c*, hydrogen peroxide; *d*, permanganate ions. *a*, 2% (wt/vol) iodine, 2.4% (wt/vol) sodium iodide. Scrapie was inactivated in both 10% and 1% suspensions of brain. Phage inactivations were in 10% brain homogenate or PBS. *b*, 0.5% (wt/vol) phenol (10% Lysol, National Laboratories, Montvale). *c*, 3% (vol/vol) hydrogen peroxide. The hydrogen peroxide reaction with brain homogenate foams making accurate volumetric sampling difficult. For this reason the volumes of the phage samples were checked gravimetrically. The increase in λ titre observed after 1 min of exposure to 3% hydrogen peroxide and brain homogenate was observed in three replicate experiments. *d*, 0.1% (wt/vol) potassium permanganate. An obvious chemical reaction as indicated by a colour change and the evolution of a distinctive odour occurs immediately on mixing permanganate with brain homogenate. Symbols: ●, scrapie; ●, ○, ΦX174am3; ■, □, PM2; ▲, △, λ; ◆, ◇, fd. Closed symbols denote inactivations in 10% brain homogenate except in *a* where they also represent 1% brain homogenate. Open symbols represent PBS.

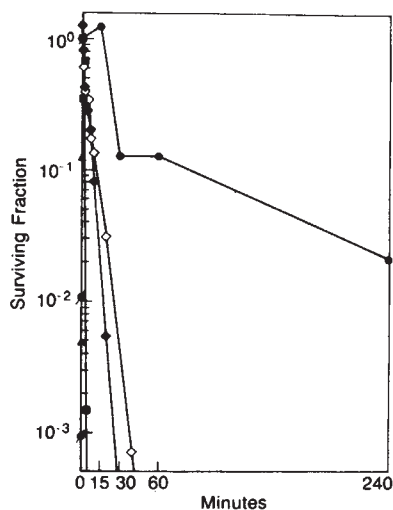


Fig. 3 Inactivation of scrapie and four control viruses by formaldehyde solution. Samples were mixed 1 : 1 with 20% formaldehyde solution F-79 (Fisher Scientific) for final concentrations of 3.7% wt/wt formaldehyde, 1 to 1.5% methanol. Symbols: ●, scrapie; ●, ○, ΦX174am3; ■, □, PM2; ▲, △, λ; ◆, ◇, fd. Closed symbols denote inactivations in 10% brain. Open symbols denote PBS.

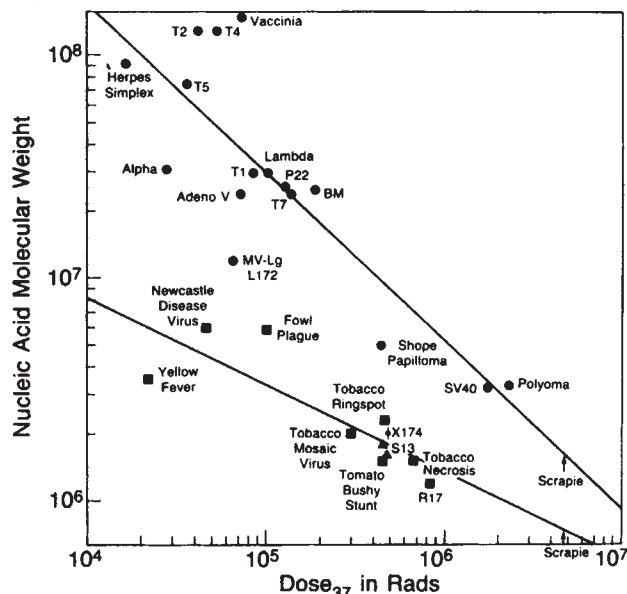


Fig. 4 Radiosensitivity of viruses as a function of the molecular weight of their nucleic acids. A literature search was conducted for all known inactivation rate constants of conventional viruses inactivated by ionizing radiation under conditions compatible with the scrapie data (first reference given below). These data, expressed as dose_{37} , the reciprocal of the inactivation rate constant, are plotted against the molecular weight of the nucleic acid component of the virus, obtained where possible from sequence or restriction enzyme data (second reference given below). ●, Double-stranded DNA viruses; ■, single-stranded RNA viruses; ▲, single-stranded DNA viruses. Separate regression lines have been drawn through the double-stranded and single-stranded virus data. References: polyoma^{9,54}, SV40^{55,56}, shope papilloma^{27,57}, phage MV-Lg-L172^{58,59}, phage T7^{27,57}, phage BM^{27,27}, phage P22^{27,57}, adenovirus 5^{27,58}, phage λ^{27,57}, phage T1^{8,57}, phage α^{60,57}, phage T5^{28,57}, herpes simplex^{8,61}, phage T2^{9,27}, phage T4^{27,27}, vaccinia^{27,62}, phage R17^{27,57}, tobacco necrosis virus^{28,28}, tomato bushy stunt^{27,27}, tomato mosaic virus^{63,27}, tomato ringspot virus^{27,64}, yellow fever virus^{8,57}, fowl plague virus^{28,65}, Newcastle disease virus^{27,57}, phage ΦX174^{27,27}, phage S13^{9,27}, scrapie (see text).

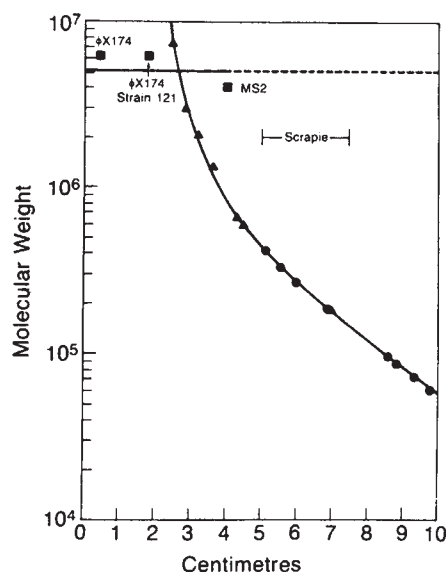


Fig. 5 Electrophoretic migration of viruses and nucleic acids as a function of their molecular weights. Three viruses of almost identical molecular weight and size (■) were co-electrophoresed with *Hind*III phage λ DNA restriction fragments (▲) and *Hae*III ΦX174 DNA restriction fragments (●) (restriction markers were obtained from Bethesda Research Laboratories). Gels were 0.5% agarose, 2.2% acrylamide, Tris acetate, pH 7.2 (4, 5). The region from which scrapie infectivity is recovered in this same gel system is indicated (ref. 4 and M. T. Borras and R.G.R., unpublished).

0.0001% of the total at 121 °C, and so cannot be used to draw structural conclusions about the agent.

The small size, 60,000–150,000 daltons of nucleic acid, inferred from scrapie's resistance to inactivation by ionizing radiation established^{7–10,23,24} and continues to foster expectations of an unconventional structure. The 'target' theory used in this calculation was intended as a mechanistic description of the physical process of inactivation by ionizing radiation²⁵; the size calculation itself depends heavily on parameters that are difficult to determine^{9,18,25}. Other than nucleic acid strandedness, the only independent variables in a size comparison between viruses are the inactivation rate constants themselves²⁵. A better size estimate can be obtained by simply comparing the scrapie inactivation rate constant directly with the inactivation rate constants of viruses of known nucleic acid size. This has been done in Fig. 4 where the log of the reciprocal of the inactivation rate constant (log dose₃₇) has been plotted against the log of the molecular weight of the nucleic acid component of all viruses for which comparable ionizing inactivation data could be found.

It is apparent from Fig. 4 that radiosensitivity of viruses is a strong function of both nucleic acid mass and strandedness^{26–29}. However, since there is considerable scatter in this relationship, it cannot be an exclusive function of these parameters as is assumed in the 'target' theory²⁵. Regression lines calculated separately for the single-stranded viruses and double-stranded DNA viruses have correlation coefficients of -0.832 and -0.841 , respectively. The scrapie inactivation rate constant, computed from the regression of six separate inactivation experiments^{7–9} over the entire dosage range of the experiments (correlation coefficient = -0.95), intercepts the single-stranded line at 0.75×10^6 daltons, well above the size of the smallest known single-stranded virus³⁰. The double-stranded line is intercepted at 1.6×10^6 daltons, well within the range of what is conceivable for a virus and 10–20 times greater than estimates made with the same data by the target calculation.

The discovery that some scrapie infectivity co-migrates with low molecular weight DNA restriction fragments and viroid RNAs in dilute agarose-acrylamide electrophoretic gels⁴ corroborated the results of the target calculation and seemingly secured the concept of subviral size. The electrophoretic gel system used in those experiments fractionated nucleic acids on the basis of their molecular weights. If scrapie were a naked nucleic acid, it must have the small molecular weight indicated by its co-migration with the viroid markers. However, if scrapie were a virus, nucleic acid markers would be inappropriate. In basic solutions, nucleic acids possess a large, uniform negative charge promoting migration, coupled with large hydrodynamic volumes and extended conformations impeding their movement through a gel matrix. In contrast, viruses are small and compact compared with the nucleic acids that they encapsulate, whereas their net charge is weak and can be negative, positive or neutral²⁹.

This difference is demonstrated in Fig. 5 where three small, icosahedral bacteriophages (MS2, Φ X174am3 and Φ X174strain 121, an electrophoretic mutant of Φ X174) of almost identical molecular weight have been electrophoresed with restriction endonuclease fragments of Φ X174 and phage λ DNA. The relative migrations of both the viruses and the nucleic acid fragments are plotted against the logs of their molecular weights. Whereas the nucleic acid fragments separate as a function of their molecular weights as predicted, virus migration is insensitive to this parameter. While none of the three test viruses migrated as far as the scrapie infectivity, there is no reason to believe that slightly more negative viruses, slightly smaller, or perhaps differently shaped viruses would not have done so.

If, as various authors have postulated, scrapie is a small infectious protein^{3,31,32} instead of a virus, it should be capable of penetrating the more concentrated acrylamide gels that are commonly used to fractionate proteins as well as the dilute agarose-acrylamide gels used to date. An adequate demonstration of this property would require the co-migration of scrapie infectivity with protein markers of known size in an elec-

trophoretic system that demonstrably excludes small highly charged viruses. To date, these criteria have not been met in any fractionation system³³ and, if scrapie has viral dimensions as predicted by the analysis of its inactivation by ionizing radiation given above, it will not be possible to do so.

There is ample evidence that scrapie aggregates readily^{2,29,34,35} and an aggregated subpopulation of the scrapie agent could appear more resistant to a given reagent or treatment than would the unit virus²⁹. This is because only a single inactivation event is required to kill a free virus particle (assuming that the inactivation of free virus proceeds by a first order process), whereas many inactivations are required (one for each virus) to kill an aggregate. The conditions in which the radiation experiments were conducted (samples were dried onto planchets) would be expected to promote aggregation which would lead to an underestimate of the inactivation rate constant and thereby the size of the virus²⁹.

Aggregation can also affect survival through the mechanism of multiplicity reactivation which has been demonstrated after virus inactivations by ionizing radiation³⁶, formaldehyde¹⁹ and nitrogen mustard³⁷, when the virus mixture was aggregated either naturally or artificially before assay. When vaccinia virus is inactivated by any of these procedures, apparent survival increases 100-fold after aggregation^{19,36,37}.

Aggregation of dispersed viruses itself mimics inactivation. Subsequent disruption of such aggregates restores infectivity and may account for reports of the reversible inactivation of scrapie³⁸. Since aggregation is a strong function of concentration, the role of aggregation can in part be surmised from the concentration dependence of an inactivation. Thus, the inactivation of scrapie infectivity by iodine (Fig. 2a) is unlikely to have been the consequence of aggregation as the more dilute suspension shows greater inactivation.

When the inactivation behaviour and size of the scrapie agent are compared directly with viral controls, there seems no justification for classifying the agent as non-viral. Studies of the scrapie agent's sedimentation behaviour^{35,39,40}, exclusion size in permeation chromatography^{33,41} and buoyant density⁴² also suggest a virus, and several ultrafiltration experiments have placed its size between 30 and 50 nm^{41,43–45}. Furthermore, electron microscopic studies of SSVE-infected brains consistently reveal a filamentous structure^{46–50} unusual for an animal virus but consistent in size and morphology with the structures of known viruses affecting plants and bacteria.

The unconventional biological properties of the subacute spongiform encephalopathies leave little doubt that this is a unique disease class, but the evidence strongly suggests that the scrapie agent, while perhaps representing a new taxon, is nevertheless, a small virus with conventional sensitivities to heat and numerous chemical inactivants.

I thank P. Brown¹² for collaboration in the chemical inactivations of scrapie presented in Figs. 1–3, E. Green for supervision of the animal colony, G. Sokol and J. Etherton for assistance in phage inactivations, M. H. Edgell and C. A. Hutchison for providing Φ X174 strain 121, E. Elliott and J. Goudsmit for critical reading of the manuscript and D. C. Gajdusek for advice and discussion.

Received 3 July 1983; accepted 1 February 1984.

- Gajdusek, D. C. *Science* **197**, 943–960 (1977).
- Millson, G. C., Hunter, G. D. & Kimberlin, R. H. in *Slow Virus Diseases of Animals and Man* (ed. Kimberlin, R. H.) 243–266 (North-Holland, Amsterdam, 1976).
- Prusiner, S. B. *Science* **216**, 136–144 (1982).
- Malone, T. G., Marsh, R. F., Hanson, R. P. & Semancik, J. S. *Nature* **278**, 575–576 (1979).
- Prusiner, S. B. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 2984–2988 (1980).
- Alper, T., Cramp, W. A., Haig, D. A. & Clarke, M. C. *Nature* **214**, 764–766 (1967).
- Alper, T., Haig, D. A. & Clarke, M. C. *Biochem. biophys. Res. Commun.* **22**, 278–284 (1966).
- Alper, T. & Haig, D. A. *J. gen. Virol.* **3**, 157–166 (1968).
- Latarjet, R. in *Slow Transmissible Diseases of the Nervous System* Vol. 2 (eds Prusiner, S. B. & Hadlow, W. J.) 387–407 (Academic, New York, 1979).
- Gibbs, C. J. Jr, Gajdusek, D. C. & Latarjet, R. *Proc. natn. Acad. Sci. U.S.A.* **75**, 6268–6270 (1978).
- Rohwer, R. G. in *Virus Non Conventiionnels et Affections du System Nerveux Central* (eds Court, L. A. & Cathala, F.) 84–113 (Masson, Paris, 1983).
- Brown, P., Rohwer, R. G., Green E. M. & Gajdusek, D. C. *J. infect. Dis.* **145**, 683–687 (1982).

13. Zlotnik, I. & Stamp, J. T. *Vet. Rec.* **77**, 1178–1179 (1965).
14. Zlotnik, I. & Stamp, J. T. *Vet. Rec.* **78**, 222 (1966).
15. Burger, D. & Gorham, J. R. *Res. vet. Sci.* **22**, 131–132 (1977).
16. Gajdusek, D. C., Gibbs, C. J. Jr, Collins, G. & Traub, R. D. *New Engl. J. Med.* **294**, 553 (1976).
17. Bernoulli, C. *et al.* *Lancet* **i**, 478–479 (1977).
18. Gard, S. & Maaloe, O. in *The Viruses* Vol. 1 (eds Burnet, F. M. & Stanley, W. M.) 359–427 (Academic, New York, 1959).
19. Kim, K. S. & Sharp, D. G. *J. Immun.* **99**, 1221–1225 (1967).
20. Burger, D., Gorham, J. R. & Leader, R. W. in *Slow, Latent and Temperate Virus Infections* (eds Gajdusek, D. C., Gibbs, C. J. Jr & Alpers, M.) 307–313 (NINDB Monogr. 2, US Government Printing Office, Washington DC, 1965).
21. Salk, J. E. & Gori, J. B. *Ann. N.Y. Acad. Sci.* **83**, 609–637 (1960).
22. Rohwer, R. G. *Science* **223**, 600–602 (1984).
23. Alper, T., Haig, D. A. & Clark, M. C. *J. gen. Virol.* **41**, 503–516 (1978).
24. Field, E. J., Farmer, F., Caspar, E. A. & Joyce, G. *Nature* **222**, 90–91 (1969).
25. Lea, D. E. *Actions of Radiations on Living Cells* (Cambridge University Press, 1946).
26. Ginoza, W. in *Methods in Virology* Vol. 4 (eds Maramorosch, K. & Koprowski, H.) 139–209 (Academic, New York, 1968).
27. Kaplan, H. S. & Moses, L. E. *Science* **145**, 21–25 (1964).
28. Terzi, M. *Nature* **191**, 461–463 (1961).
29. Rohwer, R. G. & Gajdusek, D. C. *Proc. 1st int. Symp. Herie Foundation* (ed. Boese, A.) 333–355 (Verlag Chemie, Weinheim, 1980).
30. Tischer, I., Gelderblom, H., Vettermann, W. & Koch, M. A. *Nature* **295**, 64–66 (1982).
31. Griffith, J. S. *Nature* **215**, 1043–1044 (1967).
32. Pattison, I. H. & Jones, K. M. *Vet. Rec.* **80**, 2–9 (1967).
33. Diring, H., Kimberlin, R. H. *Biosci. Rev.* **3**, 563–568 (1983).
34. Malone, T. G., Marsh, R. F., Hanson, R. P. & Semancik, J. S. *J. Virol.* **25**, 933–935 (1978).
35. Prusiner, S. B. *et al.* *Biochemistry* **17**, 4993–4999 (1978).
36. Kim, K. S. & Sharp, D. G. *Radiat. Res.* **33**, 30–36 (1968).
37. Kim, K. S. & Sharp, D. G. *J. Virol.* **1**, 45–49 (1967).
38. Prusiner, S. B. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **78**, 4606–4610 (1981).
39. Millson, G. C. & Manning, J. E. in *Slow Transmissible Diseases of the Nervous System* Vol. 2 (eds Prusiner, S. B. & Hadlow, W. J.) 409–424 (Academic, New York, 1979).
40. Prusiner, S. B. *et al.* *J. Neurochem.* **35**, 574–582 (1980).
41. Kimberlin, R. H., Millson, G. C. & Hunter, G. D. *J. comp. Path.* **81**, 383–391 (1971).
42. Rohwer, R. G., Brown, P. W. & Gajdusek, D. C. in *Slow Transmissible Diseases of the Nervous System* Vol. 2 (eds Prusiner, S. B. & Hadlow, W. J.) 465–477 (Academic, New York, 1979).
43. Eklund, C. M., Hadlow, W. J. & Kennedy, R. C. *Proc. Soc. exp. Biol.* **112**, 974–979 (1963).
44. Gibbs, C. J. Jr, Gajdusek, D. C. & Morris, J. A. in *Slow, Latent and Temperate Virus Infections* (eds Gajdusek, D. C., Gibbs, C. J. Jr & Alpers, M.) 195–202 (NINDB Monogr. 2, US Government Printing Office, Washington DC, 1965).
45. Wilson, D. R., Anderson, R. D. & Smith, W. J. *comp. Path. Ther.* **60**, 267–282 (1950).
46. Merz, P. A., Somerville, R. A., Wisniewski, H. M. & Iqbal, K. *Acta neuropath.* **54**, 63–74 (1981).
47. Merz, P. A., Somerville, R. & Wisniewski, H. M. in *Virus Non Conventiionnels et Affections du System Nerveux Central* (eds Court, L. A. & Cathala, F.) 259–281 (Masson, Paris, 1983).
48. Merz, P. A., Somerville, R. A., Wisniewski, H. M., Manuelidis, L. & Manuelidis, E. E. *Nature* **306**, 474–476 (1983).
49. Merz, P. A. *et al.* *Science* (in the press).
50. Diring, H., Gelderblom, H., Hilmert, H., Ozel, M. & Edelbluth, C. *Nature* **306**, 476–478 (1983).
51. Marsh, R. F. & Kimberlin, R. H. *J. infect. Dis.* **131**, 104–110 (1975).
52. Kimberlin, R. H. & Walker, C. A. *J. gen. Virol.* **34**, 295–304 (1977).
53. Reed, L. J. & Muench, H. *Am. J. Hyg.* **27**, 493–497 (1938).
54. Ferguson, J. & Davis, R. W. *J. Molec. Biol.* **94**, 135–149 (1975).
55. Defendi, V., Jensen, F. & Saur, G. in *The Molecular Biology of Viruses* Vol. 6 (eds Colter, J. S. & Paranchy, W.) 645–662 (Academic, New York, 1967).
56. Reddy, V. B. *et al.* *Science* **200**, 494–502 (1978).
57. Fraenkel-Conrat, H. *Compreh. Virol.* **1** (1974).
58. Drasil, V., Doskar, J., Vilkicka, S. & Tkadlec, L. *Acta virol.* **22**, 443–450 (1978).
59. Van der Eb, A. J., Van Kesteren, L. W. & Van Bruggen, E. F. J. *Biochim. biophys. Acta* **182**, 530–541 (1969).
60. Freifelder, D. *Virology* **30**, 328–332 (1966).
61. Roizman, B. & Furlong, D. *Compreh. Virol.* **3**, 229–403 (1974).
62. Becker, Y. & Sarov, I. *J. molec. Biol.* **34**, 655–660 (1968).
63. Ginoza, W. *Nature* **199**, 453–456 (1963).
64. Diener, T. O. & Schneider, I. *Virology* **29**, 100–105 (1966).
65. Hightower, L. E. & Bratt, M. A. *Compreh. Virol.* **9**, 535–598 (1977).

Secretion of a wheat α -amylase expressed in yeast

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The translocation of secretory proteins across the endoplasmic reticulum involves the recognition and cleavage of an amino-terminal extension called the signal sequence¹. The structure of signal peptides appears to be ubiquitous in having a very hydrophobic central core², so that the signal sequence in secretory proteins from one organism could possibly be recognized by the processing and transport apparatus of another. We therefore wished to investigate whether a protein, α -amylase, one of several hydrolytic enzymes secreted from the aleurone of wheat into the endosperm during germination, could be processed and secreted in an active form from the yeast *Saccharomyces cerevisiae*, secretion being dependent upon the plant signal sequence. Here, synthesis of α -amylase was by inserting a cDNA clone coding for the entire α -amylase structural gene³ into a yeast expression vector⁴. The α -amylase protein coded for by this gene fusion has the signal sequence located internally, not at the N-terminal end of the polypeptide. Nevertheless, it is processed and the processed form is secreted into the medium in an active form. There are potential industrial applications for yeast that secrete a functional α -amylase.

A previously isolated cDNA clone for a wheat α -amylase gene was inserted into a yeast expression vector as shown in Fig. 1a. The yeast expression vector pMA230 codes for the phosphoglycerate kinase (PGK) promoter as well as the first 12 amino acids of the PGK polypeptide⁴. The α -amylase cDNA was constructed using the dG/dC homopolymer tailing method and cloned into the *Pst*I site of pBR322³. The plasmid 2128 contains the entire protein-coding sequence as well as 5' and 3' untranslated regions. The insertion of the cDNA coding for the

α -amylase gene into the single *Bam*HI site of pMA230, making the plasmid 520 (Fig. 1a), creates a hybrid gene coding for a translational fusion product as shown in Fig. 1b. From the sequence of the α -amylase gene (C.M.L., unpublished results), the polypeptide synthesized will have a total of 38 extra amino acids at its N-terminal end, coded for by the PGK gene, the normally untranslated α -amylase leader (in which there are no nonsense codons), the dG/dC homopolymer tail and DNA linker fragments (see Fig. 1b). The α -amylase signal peptide is therefore not at the N-terminal end of the polypeptide.

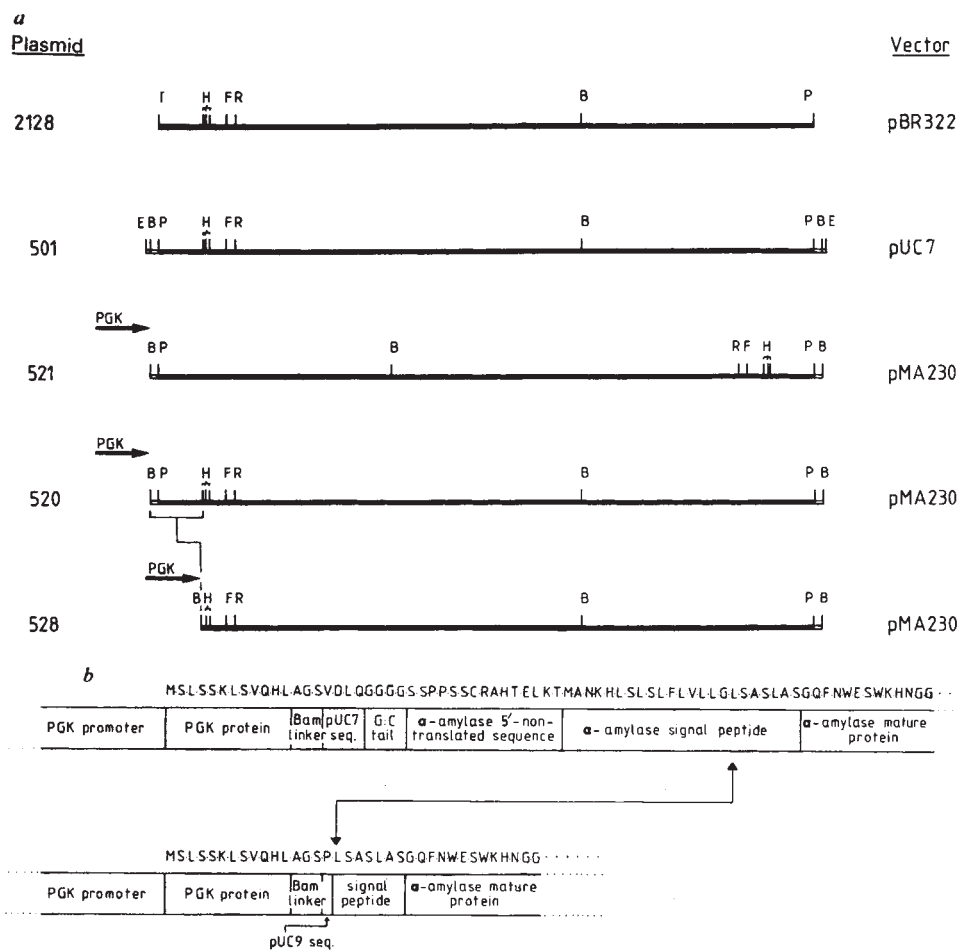
Another plasmid (528) was constructed in which the dG/dC tails, the 5' untranslated region and almost all of the signal peptide coding region were deleted (Fig. 1a). As shown in Fig. 1b, the polypeptide coded for by 528 should be 23 amino acids longer than the wild-type mature wheat α -amylase. Plasmid 521 (Fig. 1a) is the same as plasmid 520 except that the α -amylase gene was inserted in the wrong orientation to be transcribed by the PGK promoter. Yeast cells carrying this plasmid were used as non- α -amylase producing strains.

Yeast cells transformed with the plasmid 520 were labelled with ³⁵S-methionine, the intracellular protein was isolated, immunoprecipitated with anti- α -amylase serum and electrophoresed on a SDS-polyacrylamide gel. Two distinct polypeptide bands can be seen on the resulting autoradiograph shown in Fig. 2a. The larger polypeptide has an apparent molecular weight (MW) of 49,000 which is the expected value for the predicted fusion protein coded for by 520. The smaller polypeptide is the processed form of the 49,000-MW protein (S.J.R., unpublished results) and is almost identical in molecular weight to mature *in vivo* labelled wheat α -amylase (42,000 MW), although the mobilities of the two differ slightly (Fig. 2b, tracks A, B). This small difference may be caused by differences in other post-translational events such as methylation or glycosylation. When cells transformed with the plasmid 528 were labelled with ³⁵S-methionine a single polypeptide of the expected molecular weight (44,400) was immunoprecipitated by anti- α -amylase serum. As expected since the signal peptide is absent, no cleavage to a smaller protein is seen (Fig. 2c). The incorporation of label into α -amylase in cells carrying plasmid 528 is considerably greater than that seen for cells containing plasmid 520. To demonstrate that this is not due to a general increase in incorporation of the label into protein, the β subunit of the yeast mitochondrial ATPase was co-immunoprecipitated with

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Fig. 1 Plasmids constructed to analyse α -amylase synthesis in yeast.

a, A *Pst*I fragment from plasmid 2128 (ref. 3) coding for the wheat α -amylase gene was ligated into the vector pUC7 (ref. 7) to make 501. There are two *Bam*HI sites in pUC7, with a single site in the α -amylase gene (C.M.L., unpublished results). A partial *Bam*HI fragment was isolated from 501 and inserted into the single *Bam*HI site of pMA230 (ref. 4). Plasmid 520 is an insertion in the correct orientation for transcription and correct reading frame for translation of the α -amylase gene by the yeast phosphoglycerate kinase (PGK) gene, while the α -amylase gene is inverted with respect to the PGK promoter in 521. The construction of 528 involved the deletion of the dG/dC homopolymer tail, the 5' untranslated leader and most of the signal peptide as follows: there is an *Rsa*I site 302 base pairs (bp) from the *Pst*I site at the 5' end of the α -amylase sequence in 501 (C.M.L., unpublished results). This *Pst*I-*Rsa*I fragment was isolated and partially digested with *Hae*III. There are three *Hae*III sites in this fragment ~10 bp apart, with the largest *Hae*III-*Rsa*I fragment being 75 bp long. This partial digest was ligated with *Sma*I-cleaved pUC9 (ref. 7) and after transformation, a plasmid was isolated having the largest *Hae*III-*Rsa*I fragment inserted into pUC9. The restriction map of this plasmid (525) at the sequence of interest was found to be the following: *Bam*HI (from pUC9)-*Sma*I/*Hae*III (junction site)-*Hin*fl (α -amylase)-*Rsa*I/*Sma*I (junction site)-*Eco*RI (pUC9). The *Hin*fl site is 55 bp from the *Hae*III site. The reconstruction of the entire α -amylase gene involved isolating the *Bam*HI-*Hin*fl fragment from 525 and the *Hin*fl-*Eco*RI fragment from 501 and ligating the two with *Bam*HI plus *Eco*RI-cleaved pUC9 in a triple ligation to make 526. The partial *Bam*HI fragment coding for the α -amylase gene was isolated from 526, and inserted into pMA230 giving 528, which has the α -amylase gene in the correct reading frame to be translationally fused with the PGK protein. The strain MC1022 (ref. 8) was used for the transformation involving pUC7 and pUC9. MC1061 (ref. 8) was used for all other transformations. Symbols: P, *Pst*I; H, *Hae*III; F, *Hin*fl; R, *Rsa*I; B, *Bam*HI; E, *Eco*RI; →, direction of transcription of the PGK promoter. Note that the only *Hae*III and *Rsa*I cleavage sites shown are those used in the construction. **b**, Predicted α -amylase translation products coded for by plasmids 520 and 528. The amino acid sequence at the N-terminal end of the α -amylase polypeptides coded for by 520 and 528 are derived from the DNA sequences of pMA230 (ref. 4), various linker fragments (refs 4, 7) and of the α -amylase gene from 2128 (C.M.L., unpublished results). The latter sequence includes the dG/dC homopolymer tails and the 5' untranslated region as well as the normal protein coding sequence. The site of cleavage of the wheat α -amylase signal peptide is predicted from the known cleavage site of the barley enzyme whose DNA sequence in this region is identical (P. Chandler, personal communication).



α -amylase as an internal control (Fig. 2b, c). The cytoplasmic α -amylase activity in cells containing plasmid 528 is about 10 times higher than when 520 is present (data not shown). No detectable α -amylase was precipitated in cells transformed with plasmid 521.

The cleavage of the polypeptide coded for by 520 to a size similar in molecular weight to the wild-type wheat protein raised the possibility that the yeast cell was also secreting the α -amylase. To examine this, yeast cells transformed with 520 were labelled with 35 S-methionine and then incubated with non-selective medium (YPD) containing unlabelled methionine. The protein in the medium and the intracellular protein were separately reacted with anti- α -amylase serum. As Fig. 2d shows, a radioactive polypeptide of the same molecular weight as the processed α -amylase was immunoprecipitated from the medium. Since the intracellular cytoplasmic fraction possessed both unprocessed and processed forms (as well as a third polypeptide of intermediate molecular weight that was occasionally seen), while the extracellular medium fraction possessed only the processed polypeptide, the latter must have been secreted from the cell rather than appearing due to cell leakage or rupture. One interesting phenomenon is that the secretion of

the labelled polypeptides into the medium appears to be more efficient when the chase is carried out in rich medium rather than selective YGM medium.

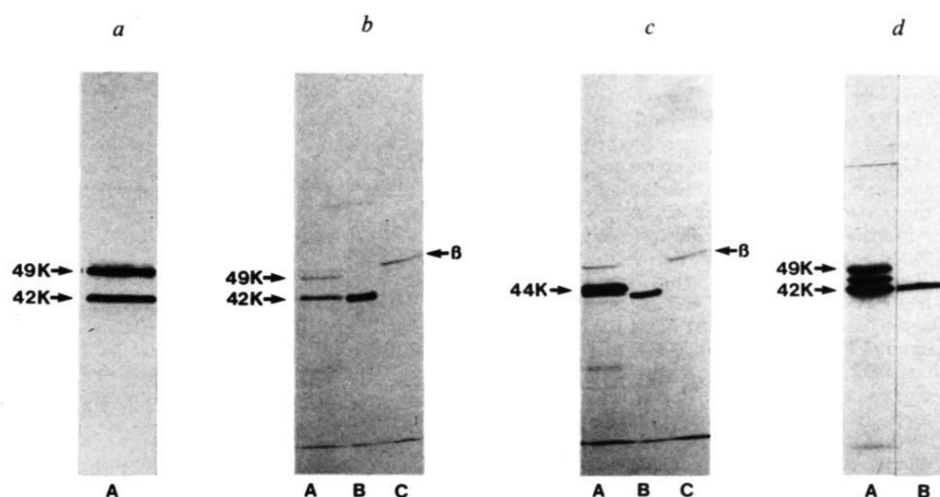
We needed to investigate whether the secreted α -amylase was functionally active. Yeast cells containing the plasmids 520, 521 and 528 were therefore streaked on YPDA plates containing 1% starch. The plates were reacted with iodine vapour which stains starch purple. As Fig. 3 shows, yeast colonies containing 520 had clear halos around them indicating degradation of the starch. There was also an occasional purple colony due to curing of the plasmid and subsequent failure to produce α -amylase. (Such colonies can arise because the plasmid was not maintained by selection on the rich medium.) In the case of yeast transformed with 528 and 521 there was no clearing, although when 528 was present the starch underneath the colony and the colonies themselves turned purple much more slowly. This is presumably due either to slight leakage of the α -amylase out of the cells or to cell rupture. There is 10 times as much α -amylase in cells carrying 528 than in those bearing 520, even though the clearing zones are far larger for the latter. These results show that the wheat α -amylase is secreted in a functionally active form. No noticeable halo was seen around cells

containing 520 plated on selective medium (YGM). This agrees with less α -amylase polypeptide being immunoprecipitated from YGM medium than from a rich medium (see above).

There are two main points about the secretion of the wheat α -amylase in yeast. First, the wheat α -amylase signal peptide is recognized by the yeast-processing apparatus even though the signal peptide is not at the N-terminal end of the polypeptide. This process is slow, however (S.J.R. *et al.*, unpublished results), presumably because the signal peptide is somewhat buried. The time taken to chase half of the labelled precursor to the mature

form was about 30–60 min. The second point is that the processed protein is secreted into the medium in an active form. The amount of enzyme secreted was variable and within the range of 30–60% of total activity. These results are likely to be of interest to the brewing industry since secretion of α -amylase from yeast cells is potentially useful in light beer production⁵. Yeast cells have already been shown to secrete animal secretory proteins⁶. The processing and export of a plant protein points to the general similarity of the eukaryotic secretory signals and processing apparatus.

Fig. 2 α -amylase polypeptides synthesized in yeast transformed with plasmids 520 and 528. The yeast cells were labelled with ^{35}S -methionine, the cells lysed, the protein immunoprecipitated with anti- α -amylase serum raised in rabbits against the purified wheat enzyme, and electrophoresed on SDS-polyacrylamide gels. **a**, 520 transformed cells labelled for 30 min with ^{35}S -methionine. **b**, Track A—520 transformed cells labelled for 30 min and then chased with unlabelled methionine for 90 min, the protein being immunoprecipitated with anti-serum against α -amylase and the β subunit of the mitochondrial ATPase; track B, *in vivo* labelled wheat α -amylase immunoprecipitated with anti- α -amylase serum; track C, labelled protein from yeast immunoprecipitated with only the anti- β -serum. **c**, Track A, 528 transformed yeast labelled for 30 min and then chased for 90 min, the protein being immunoprecipitated with both anti- α -amylase and anti- β -serum; track B, *in vivo* labelled wheat α -amylase; track C, labelled yeast protein precipitated only with anti- β -serum. **d**, Track A, cytoplasmic α -amylase protein from 520 transformed yeast labelled for 30 min with ^{35}S -methionine and then incubated with non-selective (YPDA) medium containing unlabelled methionine for 90 min; track B, the growth medium protein immunoprecipitated with anti- α -amylase serum. Numbers indicate MW ($\times 10^{-3}$).



Methods: The yeast strain used for all these experiments was Mc16 (α leu 2-3 his 4-712 ade 2-1 lys 2-1 SUF2). The yeast media used were either YPDA (a non-selective, medium containing 1% yeast extract, 2% bacto-peptone, 2% glucose and $20 \mu\text{g ml}^{-1}$ adenine) or YGM (selective medium containing 0.67% yeast nitrogen base w/o amino acids, 2% glucose and $20 \mu\text{g ml}^{-1}$ adenine, histidine and lysine). Yeast protoplasts were transformed essentially as described by Hinnen *et al.*⁹. Cells were grown to $A_{600} = 0.5$ in YGM and then labelled with 10–20 μCi ^{35}S -methionine in a volume of 200 μl . The intracellular protein was isolated by making protoplasts from the labelled yeast cells with zymolyase 60,000 in an 80- μl volume for 30 min and then lysing them by adding 20 μl 10% SDS and boiling for 3 min. After the addition of 1 ml of 1% Triton and 0.15 M NaCl, the insoluble material was removed by centrifugation for 15 min in an Eppendorf microfuge. Immunoprecipitations were carried out as previously described¹⁰. The proteins were electrophoresed on 10% SDS-polyacrylamide gels¹¹, fluoresced using the PPO-DMSO method¹² and dried for autoradiography. *In vivo* labelled wheat α -amylase was prepared by first incubating embryo-less seeds for 36 h in 20 mM sodium acetate (pH 5.5), 20 mM CaCl_2 , 0.2% chloramphenicol and 2 μM gibberellic acid. The aleurone layer was scraped away from the endosperm and 10 aleurones were incubated for 5 h with 100 μCi ^{35}S -methionine. The aleurone protein was isolated by grinding the tissue with sand in 2% SDS and then boiling for 5 min. After removing the debris, by centrifugation the α -amylase was immunoprecipitated as above.

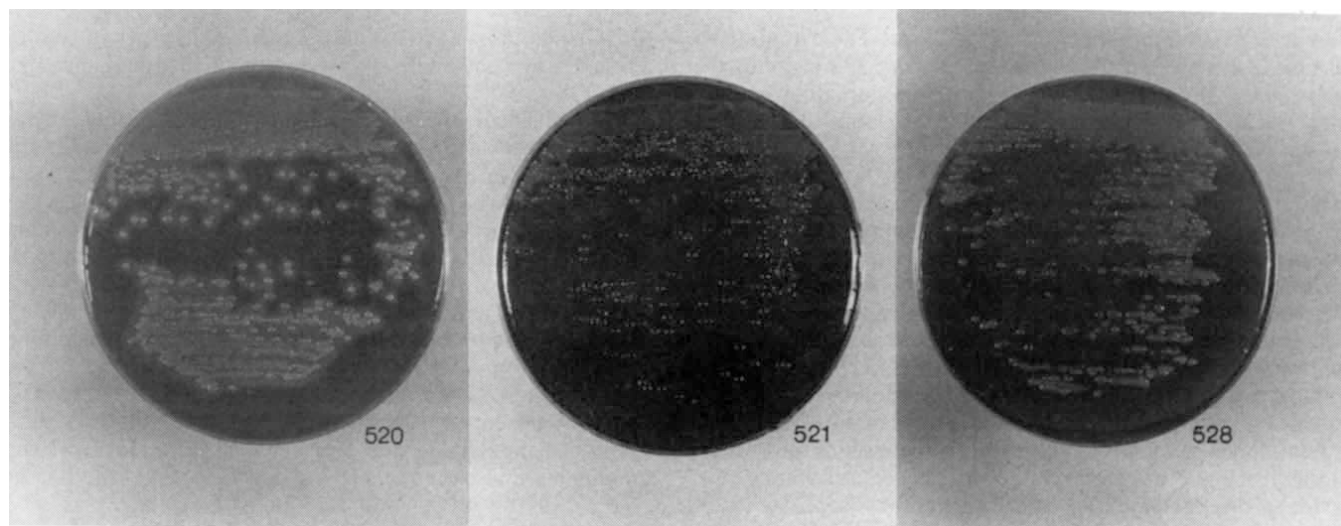


Fig. 3 Yeasts transformed with plasmids 520, 521 and 528 were streaked onto YPDA agar containing 1% soluble starch and 1 mM CaCl_2 . After colony growth, the plates were inverted onto beakers containing solid iodine, sitting in a 50 $^{\circ}\text{C}$ water bath. Clear patches indicate starch degradation, while the remaining starch turns a deep purple.

We thank M. Tuite, M. Dobson, S. Kingsman and A. Kingsman for the yeast expression vector pMA230 used in this work, and Dick Flavell for helpful discussion and reading of the manuscript. This work was in part funded by the NRDC. C.M.L. was a Shell Postdoctoral Fellow.

Received 16 November 1983; accepted 9 February 1984.

1. Blobel, G. & Dobberstein, B. *J. Cell Biol.* **67**, 835-851 (1975).
2. Finkelstein, A. V. *et al. FEBS Lett.* **161**, 177-179 (1983).
3. Baulcombe, D. C. & Buffard, D. *Planta* **157**, 493-501 (1983).
4. Tuite, M. F. *et al. EMBO J.* **1**, 603-608 (1982).
5. Tubb, R. S. *Critical Reviews in Biotechnology* (CRC Press, Boca Raton, 1983).
6. Hitzeman, R. A. *et al. Science* **219**, 620-625 (1983).
7. Vieira, J. & Messing, J. *Gene* **19**, 259-268 (1982).
8. Casadaban, M. & Cohen, S. N. *J. molec. Biol.* **138**, 179-207 (1980).
9. Hinnen, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 1929-1933 (1978).
10. Gatenby, A. A. & Castleton, J. A. *Molec. gen. Genet.* **185**, 424-429 (1982).
11. Laemmli, U. K. *Nature* **227**, 680-685 (1970).
12. Bonner, W. M. & Laskey, R. A. *Eur. J. Biochem.* **46**, 83-88 (1974).

Sequences homologous to variant antigen mRNA spliced leader in Trypanosomatidae which do not undergo antigenic variation

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Trypanosomes which parasitize mammals have evolved mechanisms to evade immune attack, such as the occupation of 'safe' intracellular sites (for example, *Trypanosoma cruzi*), or antigenic variation, exemplified by the salivarian trypanosomes (for example, *Trypanosoma brucei*). Antigenic variation is mediated by sequential expression of single variant surface glycoprotein (VSG) genes, and often involves transposition of the active gene¹⁻⁴. Every VSG transcript examined shares the same 5' terminal 35-nucleotide leader sequence^{7,8}. In *T. brucei*, this leader is encoded within a 1.4-kilobase unit tandemly reiterated to form a large array^{9,10}. It is hypothesized¹⁰ that this array is distantly linked to the expressed VSG gene and functions as a multiple promoter of VSG gene transcription, restricting transcription to that gene which, through genomic rearrangement, is placed downstream from the array. Leader and structural gene sequences are presumably juxtaposed by RNA splicing. Here we show that several trypanosomatids, both those which undergo antigenic variation (*Trypanosoma congolense* and *Trypanosoma vivax*) and those which do not (*T. cruzi* and *Leptomonas collosoma*), contain reiterated sequences homologous to the *T. brucei* spliced leader (SL). These results suggest that the SL, although utilized in VSG gene expression, is an ancestral sequence also used in the expression of other trypanosomatid genes.

We have examined several genera of Trypanosomatidae and several species of *Trypanosoma* for sequences homologous to the *T. brucei* SL by Southern analyses. Figure 1 shows restriction enzyme-digested DNAs hybridized with an end-labelled probe complementary to 22 residues of the 35 nucleotide SL. The probe hybridizes to 1.4 kilobase (kb) fragments in each of the *T. brucei* subspecies (lanes 2-4) and to a 1.5-kb fragment in the closely related species *T. equinum* (lane 1). Most of these 1.4 (or 1.5) kb units, each containing one SL sequence, are directly and tandemly repeated^{9,10}. In lanes 5-7 respectively the probe detects one major fragment in DNAs from *T. vivax* (770 base pairs, bp), *T. congolense* (840 bp) and *L. collosoma* (640 bp), while in the complete digest of *T. cruzi* DNA (lane 8) it hybridizes to a series of fragments whose sizes are multiples

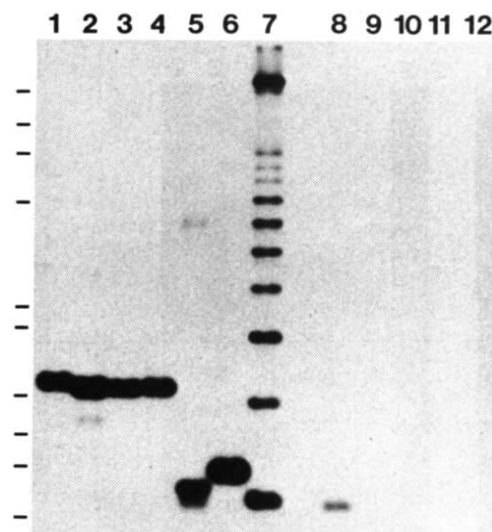


Fig. 1 Identification of genomic sequences homologous to the *T. brucei* VSG mRNA SL in several genera of Trypanosomatidae and several species of *Trypanosoma*. Lane 1, *T. equinum*, IHRI 25; lane 2, *T. b. gambiense*, Treu 1257, VAT L217; lane 3, *T. b. rhodesiense*, ILRAD 1799; lane 4, *T. b. brucei*, IsTaR 1, VAT 1.1118; lane 5, *T. vivax*, ILRAD 1819, SstII; lane 6, *T. congolense*, ILRAD 1180, PvuII; lane 7, *T. cruzi*; lane 8, *L. collosoma*, ATCC 30261; lane 9, *H. muscarum*, ATCC 30260; lane 10, *P. davidi*, ATCC 30287; lane 11, *L. tropica*, NIH strain 173; lane 12, *C. fasciculata*, ATCC 11745. The hash marks indicate λ -HindIII and Φ X174-HaeIII DNA size standards of 23, 9.6, 6.6, 4.4, 2.3, 2.0, 1.4, 1.1, 0.87 and 0.60 kb respectively.

Methods: DNA was isolated¹⁶, cleaved with the indicated restriction enzyme, size fractionated on a 1.0% agarose gel and transferred to a nitrocellulose membrane. This blot was hybridized with a synthetic 5'-[³²P]-end-labelled probe⁹ complementary to 22 of the 35-nucleotide *T. brucei* SL. Hybridization was for 18 h at 37 °C in 5 $\times$ SSPE (0.9 M NaCl, 0.05 M NaH₂PO₄, 0.005 M Na₂EDTA, pH 7.4), 0.25% Sarkosyl with 2 $\times$ 10⁶ c.p.m. of probe per ml of hybridization solution. After hybridization, the blot was washed twice at 20 °C and twice at 37 °C in the same solution. All restriction digests were with *TaqI* except where indicated.

of 650 bp. Longer autoradiography of lanes 9-12 (not shown) reveals slight but discrete hybridization to *Herpetomonas muscarum*, *Phytomonas davidi* and *Leishmania tropica* DNAs, indicating that these genomes also contain a sequence related to the *T. brucei* SL.

Surprisingly, two trypanosomatids which do not undergo antigenic variation and which are only distantly related to *T. brucei*, namely *T. cruzi* (Fig. 2a) and *L. collosoma* (Fig. 2b), show the most related organization of SL sequences. Lane 1 of Fig. 2a, b shows complete DNA digests with *BanII* and *TaqI* respectively. The SL probe hybridizes to a 650-bp fragment in *T. cruzi* and to a 640-bp fragment in *L. collosoma*. Partial digestion with these enzymes (Fig. 2a, b, lane 2) reveals a ladder of fragments whose sizes are multiples of the monomer size, demonstrating that, as in *T. brucei*, the monomers are directly and tandemly repeated. When these DNAs are digested with enzymes which do not cleave the monomer (null restriction, lane 3) hybridization is primarily to the intact tandem array, although several smaller fragments are also detected. In *T. brucei*⁹ the majority of such fragments are orphans¹¹, that is, dispersed from the array of tandem repeats.

The organization of SL sequences is more complex in *T. vivax* and *T. congolense*. Complete *SstII* digestion of *T. vivax* DNA (Fig. 2c, lane 1) produces a 770-bp fragment plus several fragments which hybridize less strongly with the SL probe. At least some of these 770-bp fragments are organized in direct tandem repeats since partial *SstII* digestion (Fig. 2c, lane 2) liberates multiples of the 770-bp unit up to the heptamer, at which point the pattern is obscured by other fragments. In addition to larger fragments, the complete *HinfI* digest (Fig. 2c, lane 3) liberates

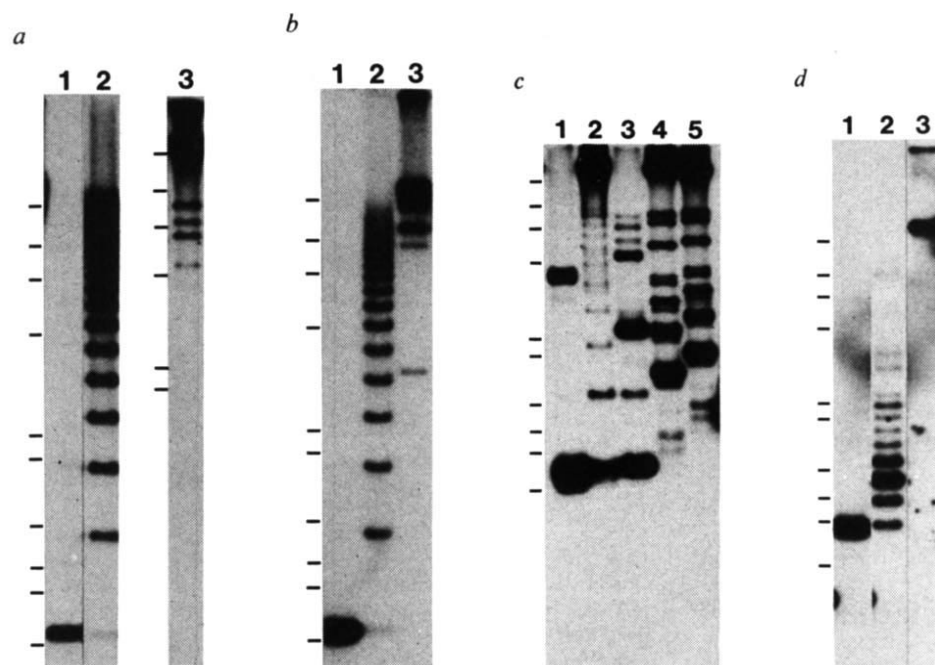


Fig. 2 Organization of sequences homologous to the *T. brucei* SL in the genomes of *T. cruzi* (a), *L. collosoma* (b), *T. vivax* (c), and *T. congolense* (d). Southern analysis using the SL probe was performed as described in Fig. 1. a, *T. cruzi* DNA (lane 1) completely and (lane 2) partially digested with *Ban*II and (lane 3) completely digested with *Bst*XI. b, *L. collosoma* DNA (lane 1) completely and (lane 2) partially digested with *Taq*I and (lane 3) completely digested with *Dde*I. c, *T. vivax* DNA (lane 1) completely and (lane 2) partially digested with *Sst*II and (lane 3) completely digested with *Hinf*I. (lane 4) *Cla*II and (lane 5) *Ban*II. d, *T. congolense* DNA (lane 1) completely digested with *Pvu*II, (lane 2) *Hae*II and (lane 3) *Hinc*II.

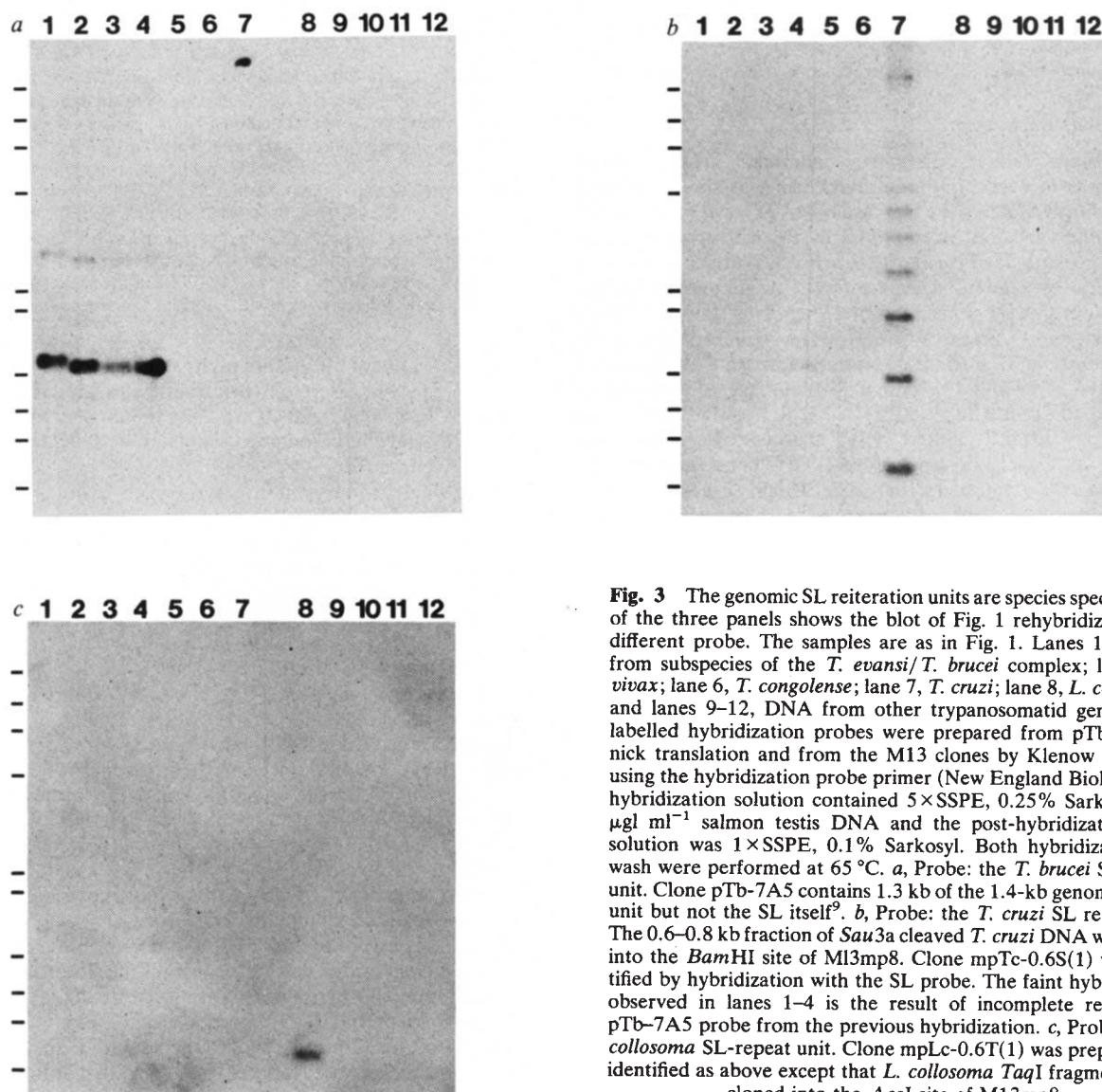


Fig. 3 The genomic SL reiteration units are species specific. Each of the three panels shows the blot of Fig. 1 rehybridized with a different probe. The samples are as in Fig. 1. Lanes 1-4, DNA from subspecies of the *T. evansi*/*T. brucei* complex; lane 5, *T. vivax*; lane 6, *T. congolense*; lane 7, *T. cruzi*; lane 8, *L. collosoma*; and lanes 9-12, DNA from other trypanosomatid genera. ³²P-labelled hybridization probes were prepared from pTb-7A5 by nick translation and from the M13 clones by Klenow extension using the hybridization probe primer (New England Biolabs). The hybridization solution contained 5×SSPE, 0.25% Sarkosyl, 200 μg ml⁻¹ salmon testis DNA and the post-hybridization wash solution was 1×SSPE, 0.1% Sarkosyl. Both hybridization and wash were performed at 65 °C. a, Probe: the *T. brucei* SL-repeat unit. Clone pTb-7A5 contains 1.3 kb of the 1.4-kb genomic repeat unit but not the SL itself⁹. b, Probe: the *T. cruzi* SL repeat unit. The 0.6-0.8 kb fraction of *Sau*3a cleaved *T. cruzi* DNA was cloned into the *Bam*HI site of M13mp8. Clone mpTc-0.6S(1) was identified by hybridization with the SL probe. The faint hybridization observed in lanes 1-4 is the result of incomplete removal of pTb-7A5 probe from the previous hybridization. c, Probe: the *L. collosoma* SL-repeat unit. Clone mpLc-0.6T(1) was prepared and identified as above except that *L. collosoma* *Taq*I fragments were cloned into the *Acc*I site of M13mp8.

monomers and dimers of the 770-bp unit indicating that some members of the tandem repeat lack *HinfI* sites. The *BanII* (Fig. 2c, lane 4) and *ClaII* (lane 5) digests produce very similar patterns of hybridizing fragments but individual fragments are offset in size by 200 bp. Thus, although some SL units in *T. vivax* are directly and tandemly repeated there is an overlaying complexity to the SL organization.

SL sequences are also reiterated in the genome of *T. congolense* (Fig. 2d). Hybridization of the SL probe to DNA digested with *PvuII* (Fig. 2d, lane 1) reveals a single 840-bp fragment. Digestion with many enzymes reveals a pattern identical to that produced by *HaeII* (lane 2). The smallest hybridizing fragment in these digests is 840 bp and each of eight larger fragments differs by additions of 190 bp. In null digests (for example, *HincII*, lane 3) the probe only hybridizes to large molecular weight DNA, suggesting that all the SL subunits may be linked.

To see if the SL reiteration units of these species share sequence homologies in addition to the SL, we rehybridized the blot of Fig. 1 with a probe containing 1.3 kb of the 1.4-kb *T. brucei* repeat unit, but not the SL itself⁹. Surprisingly, even at stringencies much lower than shown here, only the SL repeat units of the *T. brucei*/*T. evansi* subspecies hybridized (Fig. 3a). This blot was also rehybridized with probes containing the cloned SL repeats of *T. cruzi* (Fig. 3b) and *L. collosoma* (Fig. 3c). In both cases, the probes hybridized to homologous DNA only. Thus, although SL sequences are present in several trypanosomatid species, the SL reiteration unit is species specific.

The evolution of VSG sequences appears to be quite rapid¹². For example, despite cross-hybridization within the *T. brucei*/*T. evansi* complex, cDNA probes encoding 10 distinct VSGs of *T. brucei* subspecies do not hybridize to DNA of other trypanosomatids, even to the closely allied species *T. congolense* and *T. vivax* (G.N. *et al.* unpublished data). In sharp contrast, the data presented here suggest that the SL sequence is conserved among many trypanosomatids. Clear-cut hybridization of the SL probe to DNA of *L. collosoma*, a member of the most primitive genus of the Trypanosomatidae¹³, indicates that the SL sequence predates the genus *Trypanosoma*, thought to have arisen in the Devonian period 300 Myr ago.

Our findings that sequences homologous to the SL are reiterated in species which do not undergo antigenic variation implies that SL-like sequences have functions unrelated to antigenic variation and VSG expression. This hypothesis is supported by the observation that both *T. cruzi* and *L. collosoma* transcribe these sequences to produce discrete RNA size classes which hybridize with the SL probe (R.G.N. and M.S., unpublished results). In addition, the SL is heavily transcribed during the procyclic stage of the *T. brucei* life cycle when VSG transcripts and VSGs are undetectable^{10,14}. We have also isolated many non-VSG clones containing the SL (but not the 1.4-kb SL repeat unit) from *T. brucei* cDNA libraries. Unlike VSG genes, the transcription of these sequences is neither variant antigen type specific nor specific to a life-cycle stage. Thus the SL, whatever its role in VSG gene expression, also functions in the expression of non-VSG genes.

We hypothesize that SL-like sequences, or the reiterated SL units, contain regulatory signals used by many trypanosomatid genes. If the SL functions to regulate transcription, it is expected to be linked to these genes. In this regard a leader sequence partially homologous to the SL has been found directly 5' to *T. brucei* α -tubulin genes (S. Sather *et al.* unpublished results). In contrast, no SL sequences are found within 20 kb of expressed VSG genes and no VSG precursor mRNAs larger than 5 kb have been observed. Such results raise the possibility that the SL may be added onto mRNAs either during transcription, as with influenza virus, where capped 5' terminal host cell mRNA fragments are used to prime viral transcription¹⁵, or after transcription, by a novel intermolecular process. In any case, the SL might function to regulate mRNA half life, localization or translational efficiency.

We thank Dr Jack Doyle for the ILRAD trypanosome stocks,

Dr R. Gray for *T. b. gambiense*, Dr K. Stuart for *T. b. brucei* and *T. equinum*, Dr R. Locksley for *L. tropica*, Dr H. Eisen for *T. cruzi* and Ms C. Gartside for secretarial assistance. This work was supported by the Rockefeller Foundation and grants NIH AI17309 and NSF PCM 8021995 to N.A.

Received 24 October 1983; accepted 30 January 1984.

- Williams, R. O., Young, J. R. & Majiwa, P. A. O. *Nature* **282**, 847-849 (1979).
- Hoeijmakers, J. H. J., Frasch, A. C. C., Bernards, A., Borst, P. & Cross, G. A. M. *Nature* **284**, 78-80 (1980).
- Pays, E., Van Meirvenne, N., LeRay, D. & Steinert, M. *Proc. natn. Acad. Sci. U.S.A.* **78**, 2673-2677 (1981).
- Longacre, S. *et al. Molec. cell. Biol.* **3**, 399-409 (1983).
- Parsons, M. *et al. Molec. biochem. Parasit.* **9**, 255-269 (1983).
- Murphy, W. J., Bretano, S. T., Rice-Ficht, A. C., Dorfman, D. M. & Donelson, J. E. *J. Protozool.* (in the press).
- Van der Ploeg, L. H. T. *et al. Nucleic Acids Res.* **10**, 3591-3604 (1982).
- Boothroyd, J. C. & Cross, G. A. M. *Gene* **20**, 281-289 (1982).
- Nelson, R. G. *et al. Cell* **34**, 901-909 (1983).
- De Lange, T. *et al. Cell* **34**, 891-900 (1983).
- Childs, G., Maxson, R., Cohn, R. H. & Keddes, L. *Cell* **23**, 651-663 (1981).
- Frasch, A. C. C., Borst, P. & Van den Burg, *Gene* **17**, 197-211 (1982).
- Baker, J. R. in *Evolution in the Microbial World: 24th Symposium of the Society for General Microbiology* 343-366 (Cambridge University 1974).
- Parsons, M., Nelson, R. G., Stuart, K. & Agabian, N. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Plotch, S. J., Bouloy, M., Ulmanen, I. & Krug, R. M. *Cell* **23**, 847-858 (1981).
- Milhausen, M. *et al. Molec. biochem. Parasit.* **9**, 241-254 (1983).
- Gray, A. R. *Trans. R. Soc. trop. Med. & Hyg.* **66**, 263-284 (1972).
- Stuart, K. *et al. Parasitology* (in the press).

Why whip egg whites in copper bowls?

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Foams of chicken egg albumen have been an important element in Western cuisine for at least 300 yr¹; they lower the density of such otherwise ponderous preparations as soufflés and sponge cakes, and in the heat-annealed form known as meringues they support or crown various sucrose-rich mixtures². The raw protein foam is delicate and easily ruined by overbeating. Over the past 200 yr, protocols for the production of albumen foams have frequently specified the use of copper reaction vessels³⁻⁵. In keeping with the line of thought that holds culinary practice to be worthy of philosophical and scientific analysis⁶⁻⁸, we have investigated the nature and consequences of the copper protocol. We report here that copper utensils reduce the danger of overbeating albumen foams, and propose that the mechanism involves the metal-binding protein, conalbumin (ovotransferrin).

When proteins are adsorbed at the air-liquid interface of a foamed solution, the local imbalance of forces breaks intramolecular bonds; subsequent intermolecular bonding creates a protein film that stabilizes the foam⁹. However, if denaturation and coagulation proceed too far, the foam will drain liquid and collapse. Some resistance to surface denaturation is necessary if a protein is to produce a long-lived foam¹⁰. The film in foamed chicken egg white appears to involve only conalbumin, the globulins and ovomucin¹¹. Conalbumin, which is homologous to the human iron-transporting protein transferrin, binds Cu²⁺ as well as Fe³⁺ (refs 12, 13). Both metal-protein complexes are more resistant to various denaturing treatments than native conalbumin^{13,14}. The use of copper utensils to beat egg whites could lead to formation of the copper-conalbumin complex, whose stability might prevent excessive surface denaturation and rapid foam collapse.

To test this hypothesis, we first verified that copper utensils do indeed have an effect on albumen foam stability. When 60-ml samples of egg white were beaten by hand (3-4 strokes s⁻¹) in glass or unlined copper bowls, stiff peaks⁵ were obtained after 3.5-4.0 min in the former, and after 7.0 min in the latter. The

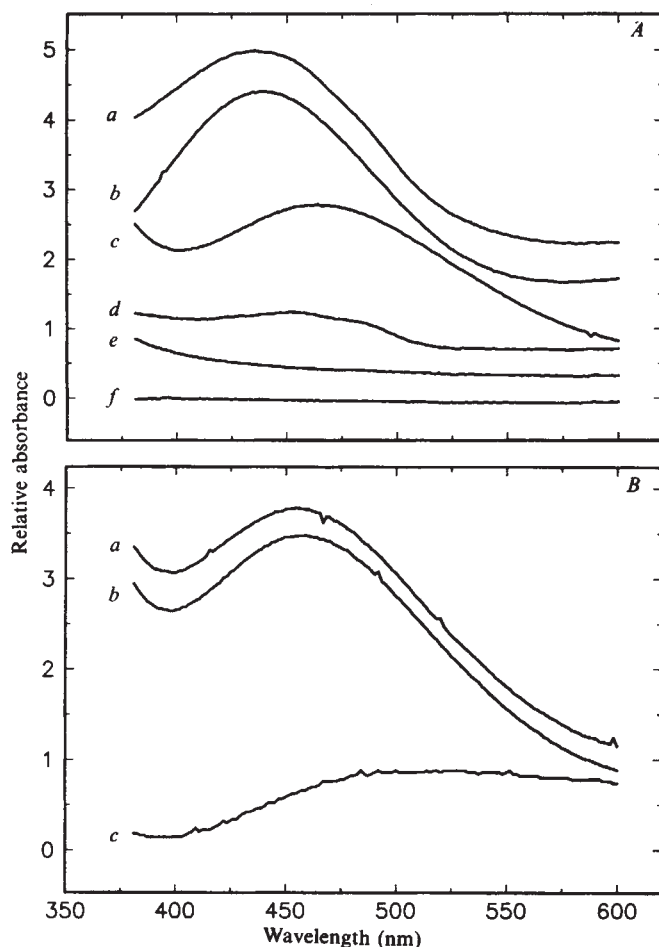


Fig. 1 The spectral behaviour of fresh egg whites beaten in various conditions. **A**, The whites of six extra large grade AA chicken eggs (Ranch Pak, San Leandro, California), pH 9.2, were mixed at 23 °C; 20-ml samples were beaten briefly (2 min) with a stainless whisk, and the clear, non-scattering liquid phase analysed with a Perkin-Elmer 356 spectrophotometer, interfaced with a Hewlett-Packard 1000 F Series computer. *a*, Unlined copper bowl (Williams-Sonoma, Palo Alto, California); *b*, glass bowl with added CuCl_2 (0.42 mM); *c*, glass bowl with added FeCl_3 (0.42 mM); *d*, glass bowl with egg white only; *e*, FeCl_3 (0.42 mM); *f*, CuCl_2 (0.42 mM). Salt concentrations were based on a calculation of the total number of metal-binding sites (two per molecule of conalbumin¹⁴) available in egg white²². The curves are displaced for clarity. All spectra were close to zero absorbance at 600 nm. As a reference, the maximum absorbance for curve *a* was near 0.5 A units. **B**, The spectral behaviour of fresh egg whites supplied with equimolar (0.42 mM) amounts of FeCl_3 and CuCl_2 . Egg white (40 ml) was beaten briefly (2 min) with the added salt solutions. A small portion of the clear, non-scattering liquid phase was analysed (curve *a*) as in **A**. The remainder was beaten to the stiff peak stage⁵, and the clear, non-scattering drainage analysed (curve *b*). Curve *c* is curve *a* subtracted from curve *b*, amplified by a factor of 4 and displaced for clarity. Maximum absorbance of curve *a* occurs at a wavelength ~9 nm lower than the iron-conalbumin peak in **A**; the maximum of curve *b* is ~6 nm lower. Curve *c* demonstrates the relative enrichment of the iron complex in the foam drainage.

sample beaten in glass became grainy within 1 min of forming stiff peaks and drained 10 ml of fluid on standing for 10 min, whereas the sample beaten 2 min past the stiff peak stage in the copper bowl had few grains, and drained only 1 ml in 20 min. Fluid loss was similarly attenuated when CuCl_2 (0.42 mM final concentration) was beaten with egg whites in a glass bowl.

Absorption spectra provided evidence for formation of the copper-conalbumin complex when egg whites were beaten in a copper bowl or in the presence of CuCl_2 (Fig. 1A). The copper-conalbumin complex is yellow and has a characteristic broad

absorption band with a maximum at 439 nm¹⁵. When FeCl_3 was added to egg whites, the reported peak at 462 nm for the salmon-coloured iron-conalbumin complex was found¹⁵. The broad, weak absorption in the spectrum of untreated egg white is probably due to a flavoprotein¹⁶, though a small amount of the iron complex may be present *ab ovo*.

In contrast to the copper-conalbumin complex, the iron-conalbumin complex is not associated with foam stabilization. Egg whites beaten with FeCl_3 (0.42 mM) into stiff peaks drained fluid rapidly (13 ml in 10 min). However, when equimolar (0.42 mM) amounts of FeCl_3 and CuCl_2 were supplied, the resulting foam was stable (<1 ml drainage in 25 min). The composite absorbance peak (Fig. 1B) produced by this mixture demonstrates that despite an estimated disadvantage of 10^{13} in dissociation constants¹⁷, Cu^{2+} binds to conalbumin in the presence of presumably saturating amounts of Fe^{3+} . It has been reported that Cu^{2+} reacts with the purified protein more rapidly than Fe^{3+} (refs 17, 18), though very rapid binding of Fe^{3+} has also been observed¹⁹.

Our hypothesis requires that while the copper-conalbumin complex is more difficult to denature than the native protein, it must still be somewhat susceptible to surface denaturation in order to participate at all in the foam-stabilizing film. One possible interpretation of the disparate effects of the two metals is that the iron complex, which is considerably more stable than the copper complex¹⁴, cannot be surface-denatured under culinary conditions, and therefore cannot play any part in foam stabilization. To test this idea, we compared the spectrum of egg whites supplied with both Fe^{3+} and Cu^{2+} with the spectrum of the drained liquid from the resulting foam (Fig. 1B). Both the difference spectrum and the shift in maximum absorbance towards the iron-complex peak indicate that the copper complex is preferentially retained in the foam.

Thus, it takes longer to beat egg whites to a given consistency in a copper bowl than it does in glass, but copper improves the foam's resistance to breakdown and so makes it more forgiving of a cook's inattention. On the basis of the spectral evidence reported here, we propose conalbumin as a possible agent for the foam-stabilizing influence of copper on egg whites. However, other albumen proteins may also be involved. Copper may react with their sulphhydryl groups to form mercaptides and so interfere with disulphide cross-bridging (P. R. Azari, personal communication), which seems to be involved in the production of egg albumen foams²⁰. Also, it is known that copper irreversibly inactivates lysozyme, one of the globulins retained in the foam, and that iron does not²¹.

We thank P. R. Azari and Joseph A. Berry for helpful discussions, and Glenn Ford for assistance with data analysis. This is publication number 832 of the Department of Plant Biology, Carnegie Institution of Washington.

Received 9 August 1983; accepted 9 February 1984.

- Phillips, E. *The New World of English Words* 6th edn (ed. Kersey, J.), S.V. Meringues (J. Phillips, London, 1706).
- McGee, H. *On Food and Cooking*, Ch. 2 (Scribner, New York, in the press).
- Diderot, D. & d'Alembert, J. (eds) *Encyclopédie, ou Dictionnaire Raisonné des Sciences, des Arts et des Métiers* 1751-1777, *Pâtissier*, Plate 1 (Briasson, Paris, 1771).
- Escoffier, A. *Guide Culinaire*, 1075 (E. Colin, Paris, 1907).
- Child, J. Bertholle, L. & Beck, S. *Mastering the Art of French Cooking* Vol 1, 158-159 (Knopf, New York, 1961).
- Plato *Gorgias*, 500e-501b (Athens, ~350 BC).
- Boswell, J. *Life of Samuel Johnson* Vol. 2, 205 (Dent, London, 1906).
- Brillat-Savarin, J. A. *Physiologie du Goût*, Méditation 7 (Santelet, Paris, 1826).
- Alexander, A. E. & Johnson, P. *Colloid Science* Vol. 2, 635-639 (Clarendon, Oxford, 1949).
- Graham, D. E. & Phillips, M. C. in *Foams* (ed. Akers, R. J.) 237-253 (Academic, London, 1976).
- Cunningham, F. E. *Poultry Sci.* **55**, 738-743 (1975).
- Fraenkel-Conrat, H. & Feeney, R. E. *Archs Biochem. Biophys.* **29**, 101-113 (1950).
- Feeney, R. E. & Allison, R. G. *Evolutionary Biochemistry of Proteins*, 144-171 (Wiley, New York, 1969).
- Azari, P. R. & Feeney, R. E. *J. biol. Chem.* **232**, 293-302 (1958).
- Komatsu, S. K. & Feeney, R. E. *Biochemistry* **6**, 1136-1141 (1967).
- Rhodes, M. B., Bennett, N. & Feeney, R. E. *J. biol. Chem.* **234**, 2054-2060 (1959).
- Warner, R. C. & Weber, I. *J. Am. chem. Soc.* **75**, 5094-5101 (1953).
- Tan, A. T. & Woodworth, R. C. *Biochemistry* **8**, 3711-3716 (1969).
- Williams, J., Evans, R. W. & Moreton, K. *Biochem. J.* **173**, 535-542 (1978).
- Johnson, T. M. & Zabik, M. E. *J. Food Sci.* **46**, 1231-1236 (1981).
- Feeney, R. E., MacDonnell, L. R. & Ducay, E. D. *Archs Biochem. Biophys.* **61**, 72-83 (1956).
- Parkinson, T. L. *J. Sci. Fd Agric.* **17**, 101-11 (1966).

From Einstein to where?

Trevor W. Marshall

In Search of Reality. By Bernard d'Espagnat.
Springer-Verlag: 1983. Pp.182. Pbk DM 42, \$16.70.

TWENTIETH-century physics has been one long sequence of unresolved questions. Are its elementary objects — light signals, atoms, electrons, quarks — particles or waves? In 1925, the definitive answer was given: "yes"! In so far as one of these objects is a particle, is it in a definite place, or can it be in several places at once? In 1927, Heisenberg plumped for the latter, but also said that an observation put it in one definite place. So what does this imply about reality? Does it mean the elementary objects of physics are not real? Or does it mean the space and time in which they exist is not real?

Physicists, especially Anglo-Saxon ones, have tended to shrug off such "metaphysical" questions, and to listen to philosophers only when they tell us we are right to shrug them off. Thus Wittgenstein ("Whereof we cannot speak, thereof we must be silent"), or the positivists of the Vienna Circle, are quoted with approval, whilst the realist statements of Karl Popper (in, for example, his *Postscript to the Logic of Scientific Discovery*) are ignored. But there were always a few physicists, including such substantial thinkers as Planck, Einstein, Ehrenfest, Schrödinger and de Broglie, who stubbornly insisted on posing these questions. With Planck and Einstein it could hardly have been otherwise, since both played crucial roles in establishing, in defence of Boltzmann, the reality of atoms, which involved a decisive rejection of Ernst Mach's positivism.

It is now a whole generation since a physicist or philosopher of such stature addressed these questions seriously. Positivist physicists have retreated into their mathematical formalism, allowing a bowdlerized version of their position to be peddled by out-and-out mystics such as Fritjof Capra (*The Tao of Physics*) and Gary Zukav (*The Dancing Wu Li Masters*). Realists have had to make do with ageing survivors of the heroic era, of whom now only Louis de Broglie and Karl Popper remain.

There is, nevertheless, some revival of interest in the ontological questions of physics. This is due in large part to John Bell's rediscovery, in 1965, of Einstein, Podolsky and Rosen's paper of 1935. These authors proposed an experimentally testable property — locality — which they considered the real objects of physics must have. As a result of Bell's efforts, experimental design has now improved to the stage where a test of this property is on the verge of being feasible.

Bernard d'Espagnat's book is a product

of this revival. Its original, French version received front-page coverage in *Le Monde*. While it deserves wider attention from the anglophone world than a brief review in *Nature*, it may confidently be predicted that it will not make the front pages of our national newspapers. In some ways this is a pity. It could well turn out that English-speaking physicists will miss some very fundamental science through their contempt for philosophy. In writing this book d'Espagnat is continuing the efforts of his *Conceptual Foundations of Quantum Mechanics*, published by Benjamin in 1976, which drew many of the ontological problems to their attention. Though certainly not easy reading, this new book addresses a much broader audience.

The book has one outstanding virtue. It says very clearly why science has a responsibility to concern itself with reality, and, therefore, why positivism, or "the philosophy of experience" as d'Espagnat calls it, is inadequate:

If the rumor were now to spread that . . . science ultimately misses reality or should not bother with it, then, undoubtedly, the portion of truth that such an assertion contains would at once be simplified and distorted by thousands of commentators . . . very happy to be able to justify some superstition or some momentary fashion [p.95].

He can say that again!

When he comes to offer a realist alternative, d'Espagnat is, however, far less convincing. This is because he is anxious to argue for a particular form of realism—non-physical realism or veiled reality — and against physical realism, represented above all by Einstein, but also by today's dominant school in the biological sciences. The latter he obviously considers particularly easy meat for his grinder, for in Chapter 5 he constructs an utterly unfair

caricature of the biological scientist operating within a materialist realism which the wise physicist discarded sixty years ago. This is a common enough theme (see for example Freeman Dyson's *Disturbing the Universe*), but most biologists understand the distinction between life and consciousness quite well, so they are a good deal humbler than this caricature suggests. Indeed, I would say that they are a good deal humbler, regarding the problem of consciousness, than d'Espagnat himself. What they do tend to reject, with good reason, is animism (the idea that every electron has a bit of consciousness), on which d'Espagnat (Chapter 10) seems notably soft.

But, finally, the main target in Bernard d'Espagnat's sights is Albert Einstein and the physical realism which he so firmly advocated. Here also there is a strong element of distortion, but it would be unkind in this case to call it caricature, because I suspect that it has been done unwittingly. The "realist" analysis of a two-body decay process, which d'Espagnat gives in Chapter 4 and Appendix 1, limits very narrowly the range of models of a local realist type. It is a comparatively easy matter to construct an experiment which refutes models from within this range. To claim, as does d'Espagnat repeatedly, that any philosophical position, let alone that of Einstein, has been thereby rendered invalid, is a gross intellectual indulgence. The point d'Espagnat has overlooked is that, if we leave the narrow domain of determinist theories, there is no difficulty at all in explaining the data obtained in the experiments so far performed. Needless to say, such experiments use real detectors, with approximately 20% efficiency, and not the ideal ones, with 100% efficiency, described by d'Espagnat.

I would conclude that Bernard d'Espagnat has made out a convincing case for going out in search of reality. But he has probably not found it because he refused to look in some of the places where it is most likely to be. □

Trevor W. Marshall is a Lecturer in Mathematics at the University of Manchester.



From *Naturkræfter*, by A. Paulsen (1879).

Heavenly light — the picture is reproduced from A. Brekke and A. Egeland's *The Northern Light*, a well-illustrated, large-format book which explores the art and science stimulated by the aurora borealis. Publisher is Springer-Verlag, price is DM108, \$42.90.

Mascara on the face of the Earth

Andrew Hill

The Pleistocene: Geology and Life in the Quaternary Ice Age.

By Tage Nilsson.

Reidel/MTP Press: 1983. Pp.650. Dfl.265, \$115, £67.25.

MY former professor of geology has been known to refer to the Pleistocene as "a geological cosmetic", a superficial and possibly deceptive covering to the meat and bones beneath. Yet although study of the deeper anatomy forms the bulk of geologists' work, there is much to be said for the surface smear. Even if the rocks of this period are in a sense superficial geologically, from the point of view of life they are more fundamental — preserving important evidence about the establishment of present-day plants and animals, and recording the increasing involvement of human beings with the ecosystem.

The Quaternary is a good instance of the historical legacy of a discipline controlling to a great extent the way evidence is collected and viewed. Penck and Bruckner's scheme of glacial and interglacials, based on the Danube, was gradually extended to a Eurocentric conception of the whole world. Work in the past few years has led to a greater appreciation of the complexity of the situation and a realization of the inadequacies of the simple traditional interpretation. The new awareness has grown principally from extensive deep-sea investigations — a part of the Earth less encumbered by classical stratigraphy and notions — but also from a more detailed examination of terrestrial evidence in Europe and elsewhere. Previously there was a tendency to believe that naming constituted understanding and knowledge, whereas more stress is now placed upon process and on the interrelationships between different classes of data.

Tage Nilsson's *The Pleistocene*, a translation and extensive revision of an earlier Swedish textbook, reflects the conventional taxonomic approach. In some ways it may be regarded as a narrative dictionary, which is not to suggest that it is

dull. On the contrary, it is an account which although terse is readable and consistently informative. Beginning with a discussion of basic concepts, the Tertiary background and developments leading to the Ice Age, and lower boundary problems, Nilsson goes on to deal with the key areas of the Alps and Italy, the British Isles and the rest of Europe, before describing other continents and the oceans. Where appropriate, each section includes a treatment of the mammalian faunas and human fossils. The sections on human palaeontology and archaeology, while accurate, are traditional and not particularly profound. There are systematic reviews of the Quaternary mammals and a classification of the vascular plants that are discussed. The book is also traditional in the sense that it has the usual European emphasis. About 250 pages are devoted to Europe, whereas Africa merits only 23, and Asia only 25. Admittedly these areas are less well documented than Europe, but even North America, where much work has been carried out, is represented by only 50 pages.

Nilsson's objects are to provide an account of progress in research and of

current problems, and a guide to the literature, the emphasis being on the stratigraphical and palaeontological aspects including the evolution of human beings and of culture. In this he largely succeeds. It would be good to have the same data integrated more analytically around questions of process and interrelationships, and in terms of hypotheses about geological, biological and climatic change; that, however, would be a different book.

As it is, *The Pleistocene* provides a good historical background, is generally up to date and contains a comprehensive bibliography of over 2,000 items. And while the illustrations sometimes serve more the needs of ornamentation than of instruction, there are a lot of them. In a text of this generality and scope specialists are bound to find points of fact or emphasis to quibble with, but taken as a whole it is an impressive achievement that should be of great value to the many geologists, biologists and other scientists who are held in fascination by the intriguing mascara on the face of the Earth. □

Andrew Hill is a Research Fellow at Harvard University.

Better spectroscopy

D.W. Turner

Introduction to Photoelectron Spectroscopy.

By Pradip K. Ghosh.

Wiley: 1983. Pp.377. £52.25, \$55.

AN EARLIER volume by Pradip Ghosh, *A Whiff of Photoelectron Spectroscopy* (Wiley, 1978), proved to be an interesting and stimulating book both for experts in the field and for those about to enter it. Though somewhat idiosyncratic in style, it was obviously the work of an enthusiast. Perhaps the present volume is to be seen both as an introduction to what is now a very broad subject and a standard research text. The author has had the opportunity to build upon his earlier volume, expanding the areas where coverage was less than full and improving the order of presentation.

After an introductory chapter, there is an account of experimental techniques which occupies some 10% of the book. It has to be said, however, that this is perhaps the least critically written chapter and does not compare with the thoroughness of some of the later sections, notably the more theoretically inclined ones. Separate chapters then deal with the principal phenomena observed in core and valence shell photoelectron spectra. The first of these topics is the more systematically treated, proceeding through core binding energies and chemical shift theories (with useful comparisons with NMR and Mossbauer chemical shifts), and a small selection of illustrative applications, to brief

mention of shake-up and shake-off processes and collective resonances.

The phenomena observed in valence photoelectron spectra are so much more complex, however, that a comparable systematic treatment has not proved possible, and instead the author has picked out some of the main areas of interest from the literature of the past 25 years. Unfortunately his summary is really quite uncritical. In consequence this is a tantalizing, stimulating and sometimes frustrating section, but one which an advanced chemistry student with a well-developed critical faculty could well be advised to read.

The second half of the book divides between discussion of application to solid surfaces and a consideration of some of the more fundamental aspects of photoionization. One of the most useful sections is that containing the references and notes, which are collected together at the end of the book, organized by chapter and topic, though these occasionally appear to be afterthoughts which could more usefully have been included in the text.

Altogether this is a useful and helpful text. It is a considerable improvement on its predecessor, but not sufficiently systematic to be treated as a standard work on the subject since there are a number of important topics which are simply omitted or only cited briefly; for example, Jahn-Teller and Renner-Teller effects, which are particularly important in valence shell photoelectron spectroscopy, are mentioned only in passing and not explained. □

D.W. Turner is Reader at the Physical Chemistry Laboratory, University of Oxford.

New in paperback

● *The Self and its Brain: An Argument for Interactionism* by Karl R. Popper and John C. Eccles (issued by Routledge & Kegan Paul, price £7.95, \$14.95). The book was originally published by Springer-Verlag in 1977, and was reviewed in *Nature* 272, 770; 1978.

● *Scientific Temperaments: Three Lives in Contemporary Science* by Philip J. Hilts (issued by Touchstone, an imprint of Simon & Schuster, New York, price \$7.95). For review see *Nature* 302, 767; 1983.

Through a world of sheep

Hans-Peter Uerpmann

Sheep and Man.

By M. L. Ryder.

Duckworth: 1983. Pp.846. £55.

THE sheep — symbolic creature of docility, sacrifice and stupidity. But our psychological assessment of this animal pays scant respect to its enormous economic importance. Not only were sheep among the first animals to be domesticated, they still sustain millions of people with their products of milk, wool and meat.

In *Sheep and Man*, Michael Ryder has produced a truly comprehensive book which covers every aspect of the historical

The first part of the book deals with prehistoric and early historic times, the second part with the period from the Middle Ages to recent times. After dealing with nomadism, transhumance and other types of husbandry systems, Ryder gives a geographical overview, starting with the countries of the Middle East, then passing through Asia, Europe and Africa to the Americas, Australia and New Zealand. Types of sheep, their products and the many different ways to obtain and handle these products are illustrated and described with all the knowledge of a scientist who has devoted most of his work to the history of this animal, and who is able to offer the reader an incredible amount of detail.

The third and final part concentrates the information under more generalized headings: husbandry and sheep products are summarized and the text concludes with a chapter on "The Sheep Legacy", including Ryder's assessment of the future direction of the sheep-man relationship. An appendix with a chronological list of illustrations covering the time between about 5000 BC and the last century is as useful as the vast bibliography, which contains more than a thousand references. An exhaustive index makes the encyclopaedic character of the book complete.

As usual with works such as this, the specialist will cavil at certain points — he will not agree with some generalizations, he may find some details superfluous and others missing. No full account of my own disagreements can be given here, but it would not seriously diminish the value of *Sheep and Man*. Even the fact that the ideas on the beginnings of sheep domestication have moved on since the pertinent chapters of the book were written, is mitigated by Ryder's complete description of the earlier views. Sometimes, however, confusion is caused when reference to new publications is made in one instance, but not in others referring to the same topic. For example, it is noted on page 14 that the mouflon is now regarded as an early escapee, as a feral animal brought to the Mediterranean islands by Neolithic man as an already domesticated animal. Later, though, the possibility is discussed that the European mouflon might be among the wild ancestors of sheep. In my view most claims of the existence of an indigenous wild sheep in Europe are highly questionable, both with regard to the stratification of the respective finds and to their identification.

It will always be the fate of books with some 800 densely printed pages that their early parts will be somewhat dated when the last word is written. Most of Ryder's book cannot become outdated, however. In its painstaking compilation of facts, *Sheep and Man* is a feat of scholarship which will itself become a major historical source. □

Hans-Peter Uerpmann is at the Institut für Urgeschichte, University of Tübingen.

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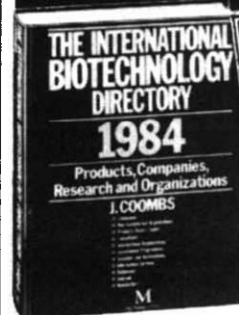
Long, long trail awinding — the traditional rotation of pastures in Switzerland, sheep returning to the valleys after spending summer grazing on the mountains.

development of this particular relationship between man and beast. For archaeologists, for historians and sociologists dealing with rural societies, for ethnologists, for farmers with an interest in history, for veterinarians and to a certain extent also for biologists, this book will be a seemingly inexhaustible mine of information about sheep and their exploitation during prehistoric and historic times. The who, what, when, where, why and how of domestication are dealt with, and the changes after domestication are covered. There is an account of the spread of sheep-husbandry from its original centre in south-west Asia towards the Mediterranean and into Europe, and also a long chapter on the sheep of the ancient civilizations.

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An omnibus for the immunologist

A.J. Munro

Fundamental Immunology.
Edited by William E. Paul.
Raven: 1984. Pp.809. \$80.

OVER the past few years there has been an explosion of information pertinent to immunologists. The advent of recombinant DNA technology, monoclonal antibodies, and the cloning of functional subsets of lymphocytes has greatly accelerated the rate at which new facts are discovered and has made possible experiments that were beer talk a few years ago.

This presents acute problems for authors or editors of textbooks, who may tackle the difficulties in one of two ways. The first is to produce a relatively short, inexpensive volume attempting a synthesis of the subject and aimed at the undergraduate. The alternative is to compete with the growing number of review journals and book series, such as *Immunology Today* and *Annual Reviews in Immunology*, and produce a more detailed, advanced textbook suitable for postgraduates. This second approach requires a firm editor to ensure uniformity of style and a balanced coverage of the subject, but the risk is that the end result will be a cumbersome book that prices itself out of markets.

Fundamental Immunology is a textbook of this latter sort. It is a big book in every sense of the word; in thirty chapters, the fifty two contributors have produced more than half a million words and over three thousand references and there are still areas of the subject that they have not discussed. Overall, the declared emphasis is on basic immunology: the molecules and cells that make up the immune system and how they interact with each other. As to be expected, there is very little discussion of any of the clinical aspects of immunology. Most of the chapters are very well written in an attractive discursive style and make sensible use of original data to illustrate key points.

The best section of the book covers the major histocompatibility complex and its role in the immune response: one of the most complicated parts of immunology. The clarity and coherence of this section may stem from the fact that all four chapters were written by colleagues working in the same institute. Some of the chapters include a few 1983 references but the bulk of the text seems to have been written in 1982 or early 1983; with such a rapidly moving subject, it is unwise for the publisher to promote the volume as "the definitive textbook" as certain chapters already look dated.

Nonetheless, there is no doubt that this book is a success. By providing in one volume a well balanced set of articles cover-

ing most aspects of basic immunology, it contributes much more to the teaching of the subject than review journals. Further, the editor has taken great pains to ensure that the book can be approached by students taking advanced courses. The only weak aspect is the index which is inadequate for a volume of this size.

My main complaint, however, is that the book costs so much and I suspect that the publisher is aiming primarily at the library market. Surely the costs could have been kept down; the book would have benefited from being 15% to 20% shorter, and is it really sensible to use such high quality paper for a volume that will be superseded in five or six years? However, the high price should not be allowed to deter libraries from buying one or several copies of this useful book. □

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Source of anomalies

Peter D. Marshall

Earthquakes, Tides, Unidentified Sounds and Related Phenomena: A Catalog of Geophysical Anomalies.

Compiled by William R. Corliss.

The Sourcebook Project, PO Box 107, Glen Arm, MD 21057: 1983. Pp.214. \$12.95.

HOW EASY it is to pick up a book, scan the title and arrive at a judgement of its contents without reading further. This could easily happen with *Earthquakes, Tides, Unidentified Sounds and Related Phenomena*. For can such a vast subject be comprehensively covered in a volume of just over 200 pages? The answer is, of course, no. But this in fact is only one of four volumes by Corliss devoted to geophysical phenomena. Eventually there will be at least 25 similar volumes cataloguing numerous "anomalies" observed in nature, all published under the auspices of the Sourcebook Project.

What Corliss is attempting to do as part of the Sourcebook Project is to scour the English-language scientific and semi-scientific literature — mainly material published in the United States and Britain over the past 150 years — to extract, assess and categorize all observed and reported anomalous phenomena with particular emphasis on those not readily explained by prevailing scientific theories. Corliss's aims are to collect and consolidate much of the unknown and poorly explained to facilitate future research and explanation. Such a task demands not only a wide scientific knowledge but considerable foresight and planning as to how the phenomena should be categorized and presented in an easily accessible form. If all succeeding volumes

of the catalogue are as well organized as this one on geophysics, then the work as a whole will satisfy these requirements.

The plan for the series is to classify each anomaly initially into subject matter (e.g. geophysical, chemical, astronomical etc.). Subsequent classifications are determined by properties directly attributable to the anomaly, e.g. sound, magnetic and so forth. A simple mnemonic lettering system (e.g. G = Geophysical) followed by a numeral is used to uniquely identify and catalogue the anomaly; this makes indexing and cross-referencing a relatively simple procedure. Once classified, the anomaly is further rated on a scale of 1 to 4 on the basis of the quality of the observations, to indicate whether, in the author's opinion, there is a high probability that the anomaly was a true phenomenon or not and whether the data are unacceptably poor. A similar scale is used to rate the quality of the explanation of the anomaly.

The phenomena catalogued in this latest volume are collected under three principal headings: the hydrosphere, earthquakes and unusual sounds in nature. All observations of water-related phenomena such as typhoon waves, geysers, tidal bores, ocean turbulence and freak wave activity are included in the hydrosphere section. The abundance of strange phenomena before, during and after earthquakes are adequately covered, though many scientists would argue with some of the conclusions presented here. Finally, the section on acoustic phenomena lists reports of strange acoustic emissions including those associated with auroral activity and winds in the desert.

For a catalogue, the book makes surprisingly interesting reading. Each phenomenon is fully described, occasionally with diagrams and figures. Additional background information, assessments and possible explanations are given as well as specific details of similar or related phenomena. Numerous observations are reported in detail with an extensive list of references dating back to the mid-nineteenth century. Such aged documentation regrettably makes verification of some observations difficult.

My main criticism of the project is one of semantics. It would have been better if, throughout the catalogue, each event was described as a phenomenon rather than an anomaly; what the author has described as an anomaly may, in reality, be a normal and predictable occurrence albeit an extremely rare one. There are inevitably other shortcomings, which may be due to a lack of consultation with appropriate experts. But on the whole Corliss has been successful in producing a book which may be of interest to both the casual browser and the specialist. □

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Chemistry

Annual Reports in Organic Synthesis — 1982. L.G. WADE Jr and M.J. O'DONNELL (eds). *Academic*: 1983. Pp. 499. Pbk ISBN 0-12-040813-9. £29.

Bimetallic Catalysts: Discoveries, Concepts and Applications. By J.H. SINFELT. *Wiley*: 1983. Pp. 164. ISBN 0-471-88321-2. £26.75, \$37.95.

Comprehensive Treatise of Electrochemistry, Vol. 7: Kinetics and Mechanisms of Electrode Processes. B.E. CONWAY et al. (eds). *Plenum*: 1983. Pp. 788. ISBN 0-306-41152-0. \$95.

Crazing in Polymers. *Advances in Polymer Science*, Vol. 52/53. H.H. KAUSCH (ed.). *Springer-Verlag*: 1983. Pp. 347. ISBN 3-540-12571-X/0-387-12571-X. DM 138, \$53.60.

Cyclophanes, Vols 1 and 2. P.M. KEEHN and S.M. ROSENFELD (eds). *Academic*: 1983. Pp. 725. Vol. 1 ISBN 0-12-403001-7; Vol. 2 ISBN 0-12-403002-5. Vol. 1 \$65; Vol. 2 \$60.

An Eight Peak Index of Mass Spectra of Compounds of Forensic Interest. By R.E. ARDREY et al. *Scottish Academic Press*: 1983. Pp. 281. ISBN 0-7073-0341-9. £50.

Guide to the Chemical Industry: Technology, R&D, Marketing, and Employment. By W.S. EMERSON. *Wiley*: 1983. Pp. 330. ISBN 0-471-89040-5. £28.95, \$40.75.

A Guide to Practical Stereochemistry. By H. ELIAS and D.M. HYDE. *Karger*: 1983. Pp. 305. ISBN 3-8055-3466-3. SwFr. 98.50, DM 118, \$39.

Mechanistic Aspects of The Thermal Formation of Halogenated Organic Compounds Including Polychlorinated Dibenzo-p-dioxins. By G.G. CHOUDHRY and O. HUTZINGER. *Gordon & Breach*: 1983. Pp. 194. ISBN 0-677-06130-7. \$39.

Modern Aspects of Electrochemistry, Vol. 15. R.E. WHITE, J.O.M. BOCKRIS and B.E. CONWAY (eds). *Plenum*: 1983. Pp. 362. ISBN 0-306-41287-X. \$49.50.

NMR Imaging: A Comprehensive Bibliography. By J. JAKLOVSKY. *Addison-Wesley*: 1983. Pp. 260. ISBN 0-201-11608-1. £28.

Organic Elemental Analysis: Ultramicro, Micro, and Trace Methods. By W.J. KIRSTEN. *Academic*: 1983. Pp. 146. ISBN 0-12-410280-8. \$23.50.

Organometallic Chemistry of Rhodium and Iridium. By R.S. DICKSON. *Academic*: 1983. Pp. 422. ISBN 0-12-215480-0. £28, \$49.

Principles of Aquatic Chemistry. By F.M.M. MOREL. *Wiley*: 1983. Pp. 446. ISBN 0-471-08683-5. £40.95, \$57.45.

Survey of Progress in Chemistry, Vol. 10. G.G. WUBBELS (ed.). *Academic*: 1983. Pp. 266. ISBN 0-12-610510-3. \$62.

Tandem Mass Spectrometry. F.W. McLAFFERTY (ed.). *Wiley*: 1983. Pp. 506. ISBN 0-471-86597-4. £37.85, \$53.15.

Three-Phase Catalytic Reactors. By P.A. RAMACHANDRAN and R.V. CHAUDHARI. *Gordon & Breach*: 1983. Pp. 427. ISBN 0-677-05650-8. \$62.95.

Transition Metal Carbene Complexes. *Verlag-Chemie*: 1983. Pp. 264. ISBN 3-527-26090-0/0-89573-073-1. DM 120.

Biological Sciences

Abusing Science: The Case against Creationism. By P. KITCHER. *Open University Press*: 1983. Pp. 213. Pbk ISBN 0-335-10199-2. £6.95.

Advances in Botanical Research, Vol. 10. H.W. WOOLHOUSE (ed.). *Academic*: 1983. Pp. 320. ISBN 0-12-005910-X. £39, \$65.

Advances in Carbohydrate Chemistry and Biochemistry, Vol. 41. R.S. TIPSON and D. HORTON (eds). *Academic*: 1983. Pp. 406. ISBN 0-12-007241-6. \$56.

Advances in Immunology, Vol. 34. F.J. DIXON and H.G. KUNKEL (eds). *Academic*: 1983. Pp. 311. ISBN 0-12-022434-8. \$39.50.

Advances in Parasitology, Vol. 22. J.R. BAKER and R. MULLER (eds). *Academic*: 1983. Pp. 416. ISBN 0-12-031722-2. £32, \$55.

Analytical Biochemistry. By D.J. HOLME and H. PECK. *Longman*: 1983. Pp. 460. ISBN 0-582-45082-9. £26.

Animal Camouflage. By L. RYLANDS. *Dinosaur Publications*: 1983. Hbk ISBN 0-85122-388-5; pbk ISBN 0-85122-387-7. Np.

Applications of Infrared, Raman, and Resonance Raman Spectroscopy in Biochemistry. By F.S. PARKER. *Plenum*: 1983. Pp. 550. ISBN 0-306-41206-3. \$65.

Avian Ecology. By C.M. PERRINS and T.R. BIRKHEAD. *Blackie/Chapman & Hall*: 1983. Pp. 221. Hbk ISBN 0-216-91478-7/0-412-00411-9; pbk ISBN 0-216-91477-9/0-412-00421-6. Hbk £19.95; pbk £9.95.

The Behavior of Gonadectomized Rhesus Monkeys. Contributions to Primatology, Vol. 20. By J. LOY, G. KEIFER and C. CONAWAY. *Karger*: 1984. Pp. 144. ISBN 3-8055-3795-6. SwFr. 66, DM 79, \$39.75.

The Behavior of Human Infants. *Life Sciences*, Vol. 13. A. OLIVERIO and M. ZAPPELLA (eds). *Plenum*: 1983. Pp. 304. ISBN 0-306-41470-8. \$42.50.

Behavioral Enrichment in the Zoo. By H. MARKOWITZ. *Van Nostrand Reinhold*: 1983. Pp. 210. ISBN 0-442-25125-4. \$24.

Biological Electron Microscopy. By B.L. GABRIEL. *Van Nostrand Reinhold*: 1982. Pp. 264. ISBN 0-442-22923-2. \$29.95.

Biological Nitrogen Fixation in Forest Ecosystems: Foundations and Applications. J.C. GORDON and C.T. WHEELER (eds). *Martinus Nijhoff*: 1983. Pp. 342. ISBN 90-247-2849-5. Dfl. 150, \$65.50.

Biological Scanning Electron Microscopy. By B.L. GABRIEL. *Van Nostrand Reinhold*: 1982. Pp. 186. ISBN 0-442-22922-4. \$24.95.

Biology and Ecology of Mangroves. H.J. TEAS (ed.). *Dr W. Junk*: 1983. Pp. 188. ISBN 90-6193-948-8. Dfl. 160, \$64.

Biology of Nonvascular Plants. By H.N. PRITCHARD and P.T. BRADT. *Times Mirror/Mosby*: 1983. Pp. 550. ISBN 0-8016-4043-1. £23.20.

The Biology of Photoreception. *Symposia of the Society for Experimental Biology*. D.J. COSENS and D. VINCE-PRUE (eds). *Cambridge University Press*: 1983. Pp. 610. ISBN 0-521-25152-4. £42.50, \$84.50.

A Biomechanical and Morphological Analysis of Human Hand Joints. *Advances in Anatomy, Embryology and Cell Biology*, Vol. 80. By J. KOEBKE. *Springer-Verlag*: 1983. Pp. 85. Pbk ISBN 3-540-12438-1/0-387-12438-1. DM 54, \$21.50.

Biotic Interactions in Recent and Fossil Benthic Communities. *Topics in Geobiology*, Vol. 3. M.J.S. TEVESZ and P.L. MCCALL (eds). *Plenum*: 1983. Pp. 837. ISBN 0-306-41292-6. \$95.

Blood Groups of Primates: Theory, Practice, Evolutionary Meaning. By W.W. SOCHA and J. RUFFIÉ. *Alan R. Liss*: 1983. Pp. 282. ISBN 0-8451-3402-7. £43.

Blood Relations: Blood Groups and Anthropology. By A.E. MOURANT. *Oxford University Press*: 1983. Pp. 146. ISBN 0-19-857580-7. £15.

Cancer in Brazil: Histopathological Data. R. RUMINI (ed.). *Ministerio da Saude*: 1982. Pp. 433. Np.

Cancer Incidence in the USSR. Supplement to *Cancer Incidence in Five Continents*, Vol. 3. N.P. NAPALKOV et al. (eds). *WHO*: 1983. Pp. 111. ISBN 92-8-321148-0. SwFr. 30, \$15.

Carbohydrate Metabolism of Tissues: Experimental and Comparative Studies. By I. KROMPECHER and M.B. LÁSZLÓ. *Akadémi Kiadó*: 1983. Pp. 215. ISBN 963-05-3229-8. \$24.

Cartilage, Vol. 1: Structure, Function, and Biochemistry. B.K. HALL (ed.). *Academic*: 1983. Pp. 385. ISBN 0-12-319501-2. \$55.

Cell Biology of the Secretory Process. M. CANTIN (ed.). *Karger*: 1984. Pp. 624. ISBN 3-8055-3619-4. SwFr. 298, DM 357, \$178.50.

Cell Locomotion In Vitro: Techniques and Observations. By C.A. MIDDLETON and J.A. SHARP. *Croom Helm*: 1983. Pp. 163. ISBN 0-7099-0324-3. £15.95.

Cells into Organs: The Forces that Shape the Embryo, 2nd Edn. By J.P. TRINKAUS. *Prentice-Hall*: 1983. Pp. 543. ISBN 0-13-121632-5. Np.

Cell Separation, Vol. 2: Methods and Selected Applications. T.G. PRETLOW II and T.P. PRETLOW (eds). *Academic*: 1983. Pp. 327. ISBN 0-12-564502-3. \$39.50.

Clinical Biochemistry of Steroid Hormones: Methods and Applications. By J.K. GRANT and G.H. BEASTALL. *Croom Helm*: 1983. Pp. 303. ISBN 0-7099-1125-4. £19.95.

Contributions to Botany: Studies in Plant Geography, Phylogeny and Evolution, Ethnobotany and Dendrological and Horticultural Botany. By H.-L. LI. *Epoch, China*: 1982. Pp. 527. \$24.50.

The Country Life Guide to Spiders of Britain and Northern Europe. By D. JONES. *Newnes*: 1983. Pp. 320. Hbk ISBN 0-600-35614-0; pbk ISBN 0-600-35665-5. Hbk £7.95; pbk £5.95.

Culture of Marine Invertebrates: Selected Readings. C.J. BERG Jr. (ed.). *Hutchinson Ross*: 1983. Pp. 386. ISBN 0-87933-105-4. \$45.

Current Methods in Cellular Neurobiology, Vol. 4: Model Systems. J.L. BARKER and J.F. MCKELVY (eds). *Wiley*: 1983. Pp. 192. ISBN 0-471-09327-0. £40.75, \$56.95.

Current Ornithology, Vol. 1. R.F. JOHNSTON (ed.). *Plenum*: 1983. Pp. 425. ISBN 0-306-41339-6. \$39.50.

A Cybernetic Approach to Colour Perception. By N.C. PARITIS and D.J. STEWART. *Gordon & Breach*: 1983. Pp. 167. ISBN 0-677-05620-6. \$29.50.

The Dandy-Walker Syndrome. By A.J. RAIMONDI, K. SATO and T. SHIMOJI. *Karger*: 1984. Pp. 80. ISBN 3-8055-1722-X. SwFr. 58, DM 69, \$34.75.

Dictionary of Life Sciences, 2nd Edn. E.A. MARTIN (ed.). *Macmillan Press, London*: 1983. Pp. 396. ISBN 0-333-34867-2. £22.50.

Die Blüte. By D. HESS. *Verlag Eugen Ulmer*: 1983. Pp. 458. ISBN 3-8001-6147-8. DM 68.

Dimensions of Darwinism: Themes and Counter-themes in Twentieth-Century Evolutionary Theory. M. GREENE (ed.). *Cambridge University Press*: 1983. Pp. 336. ISBN 0-521-25408-6. £15, \$29.95.

Disseminated Intravascular Coagulation. *Bibliotheca Haematologica*, No. 49. T. ABE and M. YAMANAKA (eds). *Karger*: 1983. Pp. 356. ISBN 3-8055-3726-3. SwFr. 197, DM 236, \$118.

Dorsal Ventricular Ridge: A Treatise on Forebrain Organization in Reptiles and Birds. By P.S. ULINSKI. *Wiley*: 1983. Pp. 284. ISBN 0-471-87612-7. £36.95, \$51.95.

Earthworm Ecology: From Darwin to Vermiculture. J.E. SATCHELL (ed.). *Chapman & Hall*: 1983. Pp. 495. ISBN 0-412-24310-5. £35.

Ecological Aspects of Radionuclide Release. Special Publication No. 3 of the British Ecological Society. P.J. COUGHTREY, J.N.B. BELL and T.M. ROBERTS (eds). *Blackwell Scientific*: 1983. Pp. 279. Pbk ISBN 0-632-01185-8. £29.80.

The Ecological Century: A Personal Appraisal. By E.B. WORTHINGTON. *Oxford University Press*: 1983. Pp. 206. ISBN 0-19-854556-8. £13.50.

The Ecological Implications of Body Size. By R.H. PETERS. *Cambridge University Press*: 1983. Pp. 329. ISBN 0-521-24684-9. £17.50, \$29.95.

Ecology and Evolution. By M.S. MANI. *Satish Book Enterprise, India*: 1983. Pp. 256. Rs. 175.

Effects of Drugs, Hormones and Chemicals on Testicular Function, Vol. 2. By A.G. DAVIES. *Eden Press*: 1983. Pp. 215. ISBN 0-88831-100-1. £29.25.

Endocrinology of Insects. R.G.H. DOWNER and H. LAUFER (eds). *Alan R. Liss*: 1983. Pp. 724. ISBN 0-84512900-7. £111.

Ether Lipids: Biochemical and Biomedical Aspects. H.K. MANGOLD and F. PALTAUF (eds). *Academic*: 1983. Pp. 439. ISBN 0-12-468780-6. \$58.

Evolutionary Biology, Vol. 16. M.K. HECHT, B. WALLACE and G.T. PRANCE (eds). *Plenum*: 1983. Pp. 499. ISBN 0-306-41408-2. \$49.50.

Fertilization in Animals. *Studies in Biology* no. 157. By B. DALE. *Edward Arnold*: 1983. Pp. 58. Pbk ISBN 0-7131-2875-5. £2.65.

Freshwater and Terrestrial Radioecology: A Selected Bibliography. By A.W. KLEMENT Jr and V. SCHULTZ. *Dowden, Hutchinson & Ross*: 1982. Pp. 587. ISBN 0-87933-389-8. \$46.

Gene Regulation by Steroid Hormones II. A.K. ROY and J.H. CLARK (eds). *Springer-Verlag*: 1983. Pp. 353. ISBN 0-387-90784-X/3-540-90784-X. DM 174, \$69.10.

The Genera of Myxomycetes. By G.W. MARTIN, C.J. ALEXOPOULOS and M.L. FARR. *University of Iowa Press*: 1983. Pp. 561. ISBN 0-87745-124-9. \$35.

Immunohistochemistry. A.C. CUELLO (ed.). *Wiley*: 1983. Pp. 501. Hbk ISBN 0-471-10245-8; pbk ISBN 0-471-90052-4. Hbk £48.95, \$79; pbk £19.95, \$35.

Immunology of Nervous System Infections. Progress in Brain Research, Vol. 59. P.O. BEHAN, V. TER MEULEN and F.C. ROSE (eds). Elsevier Biomedical/North-Holland: 1983. Pp.394. ISBN 0-444-80-443-9. \$104.25, Dfl. 245.

Immunology of Reproduction. T.G. WEGMANN, T.J. GILL III and C.D. CUMMING (eds). Oxford University Press: 1983. Pp.542. ISBN 0-19-503096-6. £30.

International Review of Neurobiology. Vol. 24. J.R. SMYTHIES and R.J. BRADLEY (eds). Academic: 1983. Pp.495. ISBN 0-12-366824-7. \$63.

Iron Binding Proteins Without Cofactors or Sulfur Clusters. Advances in Inorganic Biochemistry, Vol. 5. E.C. THEIL, G.L. EICHORN and L.G. MARZILLI (eds). Elsevier Biomedical/North-Holland: 1983. Pp.286. ISBN 0-444-00813-6. \$59.50, Dfl. 165.

Lake Chad: Ecology and Productivity of a Shallow Tropical Ecosystem. Monographiae Biologicae, Vol. 53. J.-P. CARMOUZE et al. (eds). Dr W. Junk: 1983. Pp.575. ISBN 90-6193-106-1. DG 315, \$137.

Leukotrienes and Prostacyclin. NATO Advanced Science Institutes Series, Vol. 54. F. BERTI, G. FOLCO and G.P. VELO (eds). Plenum: 1983. Pp.298. ISBN 0-306-41173-3. \$42.50.

Life in Inland Waters. By W.D. WILLIAMS. Blackwell Scientific: 1983. Pp.252. Pbk ISBN 0-86793-088-8. £9.80.

Mechanisms of Gonadal Differentiation in Vertebrates. Contributions of an EMBO Workshop held November 1982, Freiburg. U. MÜLLER and W.W. FRANKE (eds). Springer-Verlag: 1983. Pp.121. Pbk ISBN 3-540-12480-2/0-387-12480-2. DM 78, \$32.30.

Medical Histology: A Text-Atlas with Introductory Pathology. By R.L. BACON and R.R. NILES. Springer-Verlag: 1983. Pp.483. ISBN 0-387-90734-3/3-540-90734-3. DM 92, \$39.70.

Mediterranean-Type Ecosystems: The Role of Nutrients. Ecological Studies, Vol. 43. F.J. KRUGER, D.T. MITCHELL and J.U.M. JARVIS (eds). Springer-Verlag: 1983. Pp.552. ISBN 3-540-12158-7/0-387-12158-7. DM 98, \$40.50.

Metabolism of Nucleotides, Nucleosides and Nucleobases in Microorganisms. A MUNCH-PETERSEN (ed.). Academic: 1983. Pp.322. ISBN 0-12-510580-0. £31, \$52.

Methods in Enzymology. Vol. 97: Biomembranes Part K. S. FLEISCHER and B. FLEISCHER (eds). Academic: 1983. Pp.681. ISBN 0-12-181997-3. \$65.

Microclimate: The Biological Environment. 2nd Edn. By N.J. ROSENBERG, B.L. BLAD and S.B. VERMA. Wiley: 1983. Pp.495. ISBN 0-471-06066-6. £28.75, \$37.50.

Originations of Life from Volcanoes and Petroleum: A Scientific Theory Opposed to Evolution. By D.E. TYLER. Ryan Gwinner Press: 1983. Pp.128. Pbk \$10.95.

The Owl Papers. By J.E. MASLOW. Dutton: 1983. Pp.184. ISBN 0-525-24199-X. \$17.95.

Parallel Processing in the Visual System: The Classification of Retinal Ganglion Cells and Its Impact on the Neurobiology of Vision. By J. STONE. Plenum: 1983. Pp.438. ISBN 0-306-41220-9. \$55.

Physiology and Biochemistry of Tobacco Plants. By T.C. TSO, Dowden, Hutchinson & Ross: 1982. Pp.393. ISBN 0-87933-000-7. \$61.

Stream Ecology: Application and Testing of General Ecological Theory. J.R. BARNES and G.W. MINSHALL (eds). Plenum: 1983. Pp.399. ISBN 0-306-41460-0. \$55.

The Structural Basis of Neurobiology. By E.G. JONES. Macmillan Press, London: 1983. Pp.417. Pbk ISBN 0-333-36507-0. £12.

Structure and Function of Membrane Proteins. E. QUAGLIARIELLO and F. PALMIERI (eds). Elsevier Biomedical/North-Holland: 1983. Pp.378. ISBN 0-444-80540-0. \$95.75, Dfl. 225.

Structure and Function of Plant Genomes. NATO Advanced Science Institute Series, Vol. 63. O. CIFERRI and L. DURE III (eds). Plenum: 1983. Pp.495. ISBN 0-306-41346-9. \$69.50.

Sublittoral Ecology: The Ecology of the Shallow Sublittoral Benthos. R. EARL and D.G. ERWIN. Oxford University Press: 1983. Pp.277. ISBN 0-19-854573-8. £20.

Substance P, Vol. 3. Annual Research Reviews. By P. SKRABANEK and D. POWELL. Eden Press: 1983. Pp.184. ISBN 0-88831-102-8. £30.25.

Surgery of the Mind. By E.A. TURNER. Carmen Press: 1983. Pp.238. Hbk ISBN 0-946179-00-X; pbk ISBN 0-946179-01-8. Hbk £7.80; pbk £5.50.

Syllabus der Pflanzenfamilien. By A. ENGLER. Gebrüder Borntraeger: 1983. Pp.108. Pbk ISBN 3-443-02001-1. Np.

The Synergism Hypothesis: A Theory of Progressive Evolution. By P.A. CORNING. Blond & Briggs: 1983. Pp.492. ISBN 0-85634-144-X. £15.

Test Your Understanding of Neurophysiology. By R.W. MURRAY. Cambridge University Press: 1983. Pp.291. Hbk ISBN 0-521-24999-6; pbk ISBN 0-521-27127-4. Hbk £25, \$49.50; pbk £8.95, \$16.95.

Theory of Natural Selection and Population Growth. By L.R. GINZBURG. Benjamin/Cummings: 1983. Pp.160. ISBN 0-8053-3180-8. £16.95.

Tissue Culture of Trees. J.H. DODDS (ed.). Croom Helm: 1983. Pp.147. ISBN 0-7099-0830-X/0-87055-444-1. £15.95.

Vertebrate Regeneration. C.S. THORNTON and S.C. BROMLEY (eds). Dowden, Hutchinson & Ross: 1982. Pp.512. ISBN 0-87933-057-0. \$58.50.

Wildfowl 34. G.V.T. MATTHEWS and M.A. OGILVIE (eds). Wildfowl Trust, Slimbridge: 1983. Pp.176. Pbk £7, \$15.

The Wild Life of the Domestic Cat. By R. TABOR. Arrow: 1983. Pp.223. Pbk ISBN 0-09-931210-7. £3.95.

Yeast Genetics: Fundamental and Applied Aspects. J.F.T. SPENCER, D.M. SPENCER and A.R.W. SMITH (eds). Springer-Verlag: 1983. Pp.533. ISBN 0-387-90793-9/3-540-90793-9. DM 148, \$57.50.

Zinc Enzymes. T.G. SPIRO (ed.). Wiley: 1983. Pp.359. ISBN 0-471-89081-2. £73.75, \$103.45.

General

Environmental Effects of Off-Road Vehicles: Impacts and Management in Arid Regions. R.H. WEBB and H.G. WILSHIRE (eds). Springer-Verlag: 1983. Pp.534. ISBN 0-387-90737-8/3-540-90737-8. DM 132, \$52.40.

The Ethics of Environmental Concern. By R. ATTFIELD. Basil Blackwell: 1983. Pp.220. ISBN 0-631-13137-X. £17.50.

European Brewery Convention. Proceedings of the 19th Congress held London, 1983. IRL Press: 1983. Pp.728. ISBN 0-904147-50-9. £45, \$90.

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Handbook of Family and Marital Therapy. B.B. WOLMAN and G. STRICKER (eds). Plenum: 1983. Pp.487. ISBN 0-306-41228-4. \$50.

Hydrology and Water Resources in Tropical Regions. Vol. 18. By J. BALEK. Elsevier Biomedical/North-Holland: 1983. Pp.271. ISBN 0-444-99656-7/0.70.25, Dfl. 165.

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Molten Salt Techniques. Vol. 1. D.G. LOVERING and R.J. GALE (eds). Plenum: 1983. Pp.272. ISBN 0-306-41307-8. \$39.50.

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Nutritional Problems of the Elderly. Bibliotheca Nutritio et Dieta, No. 33. J.C. SOMOGYI and F. FIDANZA (eds). Karger: 1983. Pp.200. Pbk ISBN 3-8055-3700-X. SwFr. 159, DM 190, \$95.25.

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Presenting and Intervening: Introductory Topics in the Philosophy of Natural Science. By I. HACKING. Cambridge University Press: 1983. Pp.287. Hbk ISBN 0-521-23829-3; pbk ISBN 0-521-28246-2. Hbk £20, \$37.50; pbk £5.95, \$10.95.

Proceedings of the Eighth International Joint Conference on Artificial Intelligence held August 1983, West Germany, Vols 1 and 2. INTERNATIONAL JOINT CONFERENCES ON ARTIFICIAL INTELLIGENCE. Kaufmann: 1983. Pp.1206. Pbk ISBN 0-86576-064-0. £45.

Proceedings of The National Conference on Artificial Intelligence. Sponsored by the American Association, and held August 1983, Washington D.C. W.H. Freeman: 1983. Pp.442. Pbk ISBN 0-86576-065-9. £36.

The Production of Speech. P.F. MacNEILAGE (ed.). Springer-Verlag: 1983. Pp.302. 0-387-90735-1/3-540-90735-1. DM 82, \$35.40.

Prometheus. Vol. 1. University of Queensland Press: 1983. Pp.235. Np.

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Sixth ESA Symposium on European Rocket and Balloon Programmes and Related Research. held April 1983, Switzerland. European Space Agency: 1983. Pp.477. Pbk FF 140.

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